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Health Laboratory Services *in support of* Primary Health Care in South-East Asia

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Health Laboratory Services

in Support of Primary Health Care

in the South-East Asia Region

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Health Laboratory Services

in Support of Primary Health Care in the South-East Asia Region

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Foreword

The World Health Organization has been continuously striving to develop, strengthen and expand laboratory services; promote the safety and adequacy of blood and blood products and the application of appropriate and cost–effective diagnostic and therapeutic procedures that are essential for the provision of quality health care. The supportive role of laboratories is now recognized as essential for competent clinical and public health services. Utilization of laboratory services is expected in establishing accurate diagnosis and monitoring the response to treatment.

In 1994, WHO brought out the first edition of this publication to assist Member Countries in planning their national health laboratory services as well as in their implementation, development of networking of laboratories at various echelons of health services and assuring generation of quality results by the laboratories.

As with other disciplines of the sciences, health laboratory services have undergone tremendous improvements during the past five years. The second edition of the publication, with a slightly modified title, Health Laboratory Services in Support of Primary Health Care in the South-East Asia Region, is being published to provide updated information on various aspects of this discipline. This edition contains expanded and revised knowledge on rapid, sensitive and field-friendly diagnostic tests that are becoming available at affordable prices for utilization at various levels of primary health care services. The publication highlights the need to ensure biosafety and describes in detail various effective measures that have to be instituted to guarantee risk-free operation of laboratories. It also dwells in detail about the establishment of a quality assurance programme for blood transfusion services in developing countries to ensure safe blood.

Though the revised edition contains the latest technical information, the emphasis continues to be on contemporary requirements of the countries of the South-East Asia Region that are vigorously pursuing their efforts in rationalizing and strengthening health laboratory services.

The first edition of this publication received appreciation from all quarters, and I am sure that the second edition will also go a long way in disseminating appropriate technical information on the health laboratory services that have assumed a vital role in quality primary health care.

Dr Uton Muchtar Rafei
Regional Director

Preface to the First Edition

The World Development Report 1993 (Investing in Health) refers to provision of 'essential clinical services and health packages' in the countries, and Essential Health Technology is regarded as one of the main ingredients of these packages.

The major thrust of WHO in the 1990s is based principally on the concept of Essential Health Technology (EHT) and on making this accessible to the people. The essential component of EHT is promotion of health through prevention and control of prevalent diseases, for which diagnosis is an important activity and needs to be provided at the basic health care level. The delivery of diagnostic technology in an appropriate manner for public health care has to be through the establishment and development of a well-planned and executed network of health laboratories in the Member Countries.

Experience gained in the execution of a UNDP project 'Strengthening of Health Laboratories in Support of Primary Health Care' underlined the paucity of literature in this regard. As a result, this book was written with the help of WHO consultants who participated in the project.

The material for 'Health Laboratory Services in Support of Primary Health Care in Developing Countries' is presented in three parts.

Part I deals with the establishment and development of national health laboratory services. Appropriate guidelines have been given for the structure, management, functions and scope of activities of health laboratories in the countries.

Part II deals with the rapid/simple diagnostic technology available today in laboratory medicine. In order to tailor this technology to the needs of peripheral laboratories in developing countries, it is essential that the technology is transferred to the countries so that it becomes cost effective and affordable. The impact of simple diagnostic technology will be felt in the early diagnosis, therapy and control interventions of common diseases, in reduction of the disease burden and in the promotion of health of the communities.

Part III of this publication focuses on the importance of introducing, managing and sustaining quality assurance in the countries. The principles and management of quality assurance programmes are presented in detail for clinical chemistry, clinical microbiology and haematology.

Biosafety in laboratories is essential for protecting workers as well as patients and the general public, as the majority of laboratories deal with potentially infectious materials. An addendum on biosafety describes basic guidelines for the safety measures which can be taken.

Preface to the Second Edition

The Regional Publication No. 24 entitled 'Health Laboratory Services in Support of Primary Health Care in Developing Countries' was published in 1994 and incorporated the latest knowledge up to the middle of 1994. Since then, considerable advances have been made in the fields of rapid diagnostics and the methodology of quality assurance in laboratory medicine.

While not many changes have occurred in the contents of Part I, small revisions have been incorporated in the text, following the feedback received by the Regional Adviser during her visits to the countries of the Region.

As regards the contents of Part II, several advances have taken place in the development of rapid diagnostic methods for infectious diseases. In addition, emerging infections have created a need for both global alert and global collaboration in surveillance and control of such infections. Many tests which are rapid and simple have been developed for the identification of the most important emerging and re-emerging infections, and could be used in the periphery.

In Part III, updated information on the methodology of Quality Assurance in Laboratory Medicine has been incorporated. Accreditation of laboratories is now being made mandatory in many countries of the Region. Accreditation is a procedure by which an authoritative body gives formal recognition that a laboratory is competent to carry out specific tasks. Some countries have made it mandatory for laboratories to be inspected and accredited before they can start operating. Guidelines for carrying out inspections for granting accreditation have been incorporated. These guidelines have been taken from the Clinical Pathology Accreditation Ltd. (CPA), UK, and may be modified by countries as per their needs and conditions.

With the advent of AIDS, quality assurance (QA) in blood banks has become a dire necessity, especially in the context of bloodborne infections. A chapter on quality assurance for blood banks has, therefore, been added.

The phenomenal rise in the incidence of AIDS, hepatitis, tuberculosis and other emerging infectious diseases poses increased risk for laboratory workers handling clinical specimens. With a view to safeguarding laboratory personnel, the chapter on 'Biosafety in Laboratories' has been revised and updated and included as Part IV.

The title of this publication has been changed as it has been written keeping in mind the SEAR situation and its priority needs. However, it may be applicable for other developing countries as well.

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PART IV

Biosafety in Laboratories

Abbreviations and Terms Used

AAFB	Acid–alcohol-fast bacilli
ACD	Acid citrate dextrose
AFB	Acid-fast bacilli
AFP	Alpha fetoprotein
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase (also SGPT)
APTT	Activated partial thromboplastin time
ASO/ASLO	Antistreptolysin O
AST	Aspartate aminotransferase (also SGOT)
ATCC	American Type Culture Collection
BIS	Bias Index Score
BOD incubator	Biological Oxygen Demand incubator
Ca	Calcium
CCV	Chosen Coefficient of Variation
CEA	Carcinoembryonic Antigen
CFU	Colony forming unit
CIE	Counter immunoelectrophoresis
CK	Creatine kinase
CMV	Cytomegalovirus
CO_2	Carbon dioxide
CoA	Coagglutination
CPD	Citrate phosphate dextrose
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CV	Coefficient of variance
DLC	Differential leucocyte count
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
DV	Designated (target) Value
EBV	Epstein–Barr virus
EDTA	Ethylenediamine tetra-acetate (anticoagulant)
EHT	Essential health technology
EIA/ELISA	Enzyme linked immunosorbent assay
EPI	Expanded Programme of Immunization (now Global Programme on Vaccines—GPV)
ESR	Erythrocyte sedimentation rate
EQAS	External Quality Assessment Scheme
EQA	External Quality Assessment
FNAC	Fine needle aspiration cytology
FTA-ABS	Fluorescent treponemal antibody-absorbed
GLC	Gas liquid chromatography
GLP	Good laboratory practices
GMP	Good manufacturing practices
GPA	Global Programme on AIDS
GPV	Global Programme on Vaccines
G6PD	Glucose-6-phosphate dehydrogenase
Hb	Haemoglobin
HbS	Haemoglobin S (Sickle)
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCG	Human chorionic gonadotrophin
HCV	Hepatitis C virus
HCW	Health care worker
HDL	High density lipoprotein
HEPA filter	High-efficiency particulate air filter
HEV	Hepatitis E virus
HFA 2000	Health for All by the Year 2000
HiCN	Haemiglobincyanide
HIV	Human immunodeficiency virus
HRP	Histidine-rich protein
HSV	Herpes simplex virus

HTLV	Human T-cell lymphotropic virus
ICA	Immunochromatographic assay
ICSH	International Council for Standardization in Haematology
IEQAS	International External Quality Assessment Scheme
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Infectious mononucleosis
In situ	In natural or normal position
In vitro	In an artificial environment in a glass vessel
In vivo	Within living body
IQC	Internal quality control
IU	International units
JE	Japanese encephalitis
K	Potassium
KCN	Potassium cyanide
KOH	Potassium hydroxide
LA	Latex agglutination
LDL	Low density lipoprotein
LE cell	Lupus erythematosus cell
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MD	Doctor of Medicine
mg	Milligram (one-thousandth of a gram)
Mg	Magnesium
mL	Millilitre (one-thousandth of a litre)
MLT	Medical laboratory technology
mmol	Millimolar concentration
MP	Malarial parasite
MRBIS	Mean Running Bias Index Score
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRVIS	Mean Running Variance Index Scores
MSc	Master of Science
Na	Sodium
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCCLS	National Committee on Clinical Laboratory Services (USA)
NCTC	National Collection of Type Cultures (UK)
NEQAS	National External Quality Assessment Scheme
NIH	National Institutes of Health (USA)
OMRVIS	Overall Mean Running Variance Index Scores
ONPG	O-nitrophenyl (D-galactopyranoside (biochemical test for enterobacteriaceae)
Pap Smear	Papanicolaou smear (for vaginal cytology)
PCR	Polymerase chain reaction
PCV	Packed cell volume
Pf	*Plasmodium falciparum*
PFU	Plaque forming unit
PGL-1	Phenolic glycolipid-1 (species specific antigen of *Mycobacterium leprae*)
pH	Symbol for value used to denote acidity and alkalinity
PHC	Primary Health Care
PhD	Doctor of Philosophy
PPA	Phenylpyruvic acid
QA	Quality assurance
QC	Quality control
RBC	Red blood cells
RIA	Radio immunoassay
RNA	Ribonucleic acid
RPHA	Reverse passive haemagglutination
RPR	Rapid plasma reagin card
RSV	Respiratory syncitial virus
SD	Standard deviation
SDBIS	Standard Deviation of Bias Index Score
SDD	Standard deviation difference
SGOT	Serum glutamate-oxaloacetate transaminase (also AST)
SGPT	Serum glutamate-pyruvate transaminase (also ALT)
STD	Sexually transmitted disease

STS	Standard tests for syphilis
TB	Tuberculosis
TLC	Total leucocyte count
TORCH	Toxoplasma rubella CMV herpes
TPHA	*Treponema pallidum* haemadsorption test (specific treponemal antibody test)
TPS	Tissue polypeptide specific antigen
TRUST	Toluidine red unheated serum test
TSI	Triple sugar iron
µ	Micron (micrometre) (one-thousandth of a millimetre)
VDRL	Venereal Disease Research Laboratory
VI	Variance Index
VIS	Variance Index Scores
WBC	White blood cells
WHO	World Health Organization

AFRO—African Regional Office

AMRO/PAHO—American Regional Office/Pan American Health Organization

EMRO—Eastern Mediterranean Regional Office

EURO—European Regional Office

HQ—Headquarters

SEARO—South-East Asia Regional Office

WPRO—Western Pacific Regional Office

Part I

Policy Guidelines for Health Laboratory Services in the South-East Asia Region

1. Introduction

THE COUNTRIES OF the South-East Asia Region are committed to the goal of achieving Health for All by AD 2000. The approach adopted for achieving this goal is the successful delivery of primary health care (PHC).

One of the important activities at the PHC level is disease surveillance. Laboratory-based disease surveillance is far more sensitive and accurate than clinical examination. This plays a pivotal role in control activities, including vaccination programmes, administration of antimicrobial agents and control of environmental risk factors in the community. Thus, strengthening laboratory services at the PHC and the immediate supporting level of the health system is essential.

Laboratory support at the PHC level in the countries of the South-East Asia Region is not well developed due to the shortage of laboratory personnel; lack of networking between laboratories of different echelons; lack of continuing education programmes for refresher training of laboratory technicians or technologists; and lack of quality assurance programmes. The lack of laboratory-based information at the PHC level severely hampers effective therapy, disease surveillance and control, and recognition of epidemics or unusual infections.

Disease surveillance is one of the important activities at the PHC level. Laboratory-based disease surveillance is far more sensitive and accurate than clinical examination and plays a pivotal role in control activities, including vaccination programmes, administration of antimicrobial agents and control of environmental risk factors in the community.

In the countries of the Region, maximum morbidity and mortality result from: (a) *communicable diseases*, including diarrhoeal diseases, acute respiratory infections, meningitis, poliomyelitis, tuberculosis and malaria; and (b) *non-communicable diseases*, including diabetes, hypertension, liver disease, cancer, anaemia and coronary artery disease.

Laboratory-based diagnosis of these diseases at the peripheral level will be favourable both for the patient and the community. Thus, meaningful laboratory services at the periphery will lead to improvement in the quality of health care and disease surveillance and control, and, above all, will generate confidence in both the user and the health care worker.

New approaches to the control of communicable diseases are being introduced at the PHC level. The introduction of rapid/simple diagnostic methods leads to timely therapeutic intervention. Subsequent to improvement in PHC,

Establishment of national health laboratory services will allow effective monitoring of various vertical national health programmes, quality of water and food, and epidemiological investigations and will help in laying a solid foundation for control of diseases.

decentralization of some testing procedures to the primary care level, establishment of a functioning referral system and the introduction of tests for real needs and priorities, as dictated by rationality, available resources and changes in mortality and morbidity, will take place. With the availability of many simple and rapid procedures for diagnosis and determination of epidemiological markers, it has become possible to obtain laboratory-based data at the peripheral level.

This necessitates the development of a comprehensive laboratory policy, particularly in the South-East Asia Region, where networking of laboratories at different levels is weak. In most countries of the Region, the network of health laboratory services comprises: a central laboratory usually located in the capital city; intermediate level laboratories; and peripheral laboratories, which include those in first referral hospitals and those attached to health centres. There is an urgent need for equitable distribution of funds to every level of the laboratory services for effective laboratory networking. Establishment of national health laboratory services will allow effective monitoring of the quality of water and food, various vertical national health programmes and epidemiological investigations, and will help in laying a solid foundation for control of diseases. This reorganization will directly benefit a large majority of people living in rural and remote areas by bringing simple, quick and reliable diagnostic tests to their doorsteps.

The emergence and re-emergence of communicable diseases in the 1990s have focused attention on the need for their timely recognition. A WHO-coordinated international collaboration for rapid response to detect and control emerging and re-emerging infections was launched in 1996. An optimally functioning surveillance system with appropriate laboratory support can provide an early warning system leading to rapid intervention for control of epidemics/outbreaks.

2. Scope and Functions of National Health Laboratory Services

NATIONAL HEALTH laboratory services should cover all the requirements of curative and preventive medicine. The primary functions of health laboratory services are to

- assist in early and reliable diagnosis and treatment;
- investigate outbreaks of disease;
- collect reliable surveillance data for effective disease control;
- monitor the quality of water and food; and
- monitor the various vertical national health programmes, if possible.

In countries of the South-East Asia Region, the most important health problems are communicable and parasitic diseases; therefore, microbiology and hygiene (water and food) laboratories are essential. Other important laboratory disciplines include haematology,

immunology, biochemistry (clinical chemistry), histopathology and entomology.

Many other laboratory disciplines concerned with human health (such as those concerned with blood transfusion) have not been included here as they are organized separately. Other laboratories such as environmental, biophysical (for detection of radioactivity), and nutritional (for assessing dietetic deficiencies, and nutritional values of foods), and those concerned with the production of biologicals, quality control of pharmaceutical preparations, and forensic services (medico-legal and toxicological issues) may be separated or integrated with the health laboratory services, depending on the arrangements in each country. However, close coordination of the health laboratory services with all other types of laboratories concerned with human health is essential.

Health laboratory services should also be coordinated with veterinary services and sanitary engineering service laboratories. Since many communicable diseases affect both man and animals and since the laboratory disciplines of medicine and veterinary sciences are similar, close collaboration and coordination between the health laboratory and veterinary services is advantageous.

An important function of the national health laboratory services is to develop and sustain teaching and training programmes for personnel employed in the service and paramedical workers employed in the peripheral health care system. Health laboratory services should give expert advice in solving local and national health problems, and therefore a programme of research constitutes an important activity for national health laboratory services.

Epidemiological Services

Communicable diseases are the leading causes of morbidity and mortality in developing countries. There is an urgent need to give high priority to disease prevention and

Epidemiological Studies

Epidemiological surveillance

Collection and evaluation of epidemiological data and dissemination of epidemiological information to the health service is very crucial in control programmes. Sero-surveillance studies on endemic diseases are essential components of planning, forecasting and preventive programmes.

Epidemiological investigations of outbreaks of diseases in the community

The sources and routes of transmission and other factors responsible for the outbreaks may be determined by these investigations. This will give direction in devising control programmes.

Studies on the changing patterns of diseases

Details of the routes of transmission of diseases and the immunological responses of populations (in the case of infectious diseases) are essential for undertaking effective control measures.

Evaluation of different prophylactic measures in the community

This requires close coordination and cooperation between epidemiological and health laboratory services.

Studies on the epidemiology of noncommunicable diseases in the population

Close coordination and cooperation is needed between clinicians, epidemiologists and laboratory scientists.

control programmes for communicable diseases to reduce their incidence. The control of these diseases requires a multidisciplinary approach involving epidemiologists, laboratory scientists, clinicians, public health specialists and health statisticians. In this regard, epidemiological studies must be closely integrated with health laboratory services.

It is now accepted that epidemiology is no longer limited to disease surveillance and control. Epidemiological research can contribute much to the development of PHC through the identification and selection of problems as well as the understanding of their causes, suggesting solutions, implementing changes and evaluating solutions. Only those aspects of epidemiological studies that can be coordinated with health laboratory services are included here.

Most personnel employed in the clinical laboratories of the Region lack knowledge of the basic public health principles governing epidemiologic research in disease surveillance. Therefore, it is essential that principles of epidemiology and public health are included in the training programmes. Epidemiological services can be fully integrated with the health laboratory services or can form an independent service, depending on the health infrastructure in the country.

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Environmental Pollution and its Hazards

In recent years, due to industrialization and other advances, a very dangerous situation has arisen —pollution of the environment, threatening life on earth. There is large-scale pollution of the air with chemicals, fumes and waste products from automobiles and industries. Pollution of water with heavy metals, pesticides and sewage is widespread. Pollution of soil with pesticides and insecticides is common, and contaminates food from agricultural and animal sources.

Detection and monitoring of all these pollutants is complex, highly specialized and very expensive. According to their circumstances, countries of this Region have to decide whether to add this considerable load of work to the health laboratory services or whether such laboratories should be separate. In many developing countries the two services are separate.

Countries, where the central and regional (provincial) laboratories are equipped to detect and monitor environmental pollution and its hazards, should include a programme of education for health staff at lower echelons to recognize and promptly refer suspected cases identified.

Another aspect of environmental pollution involves the transmission of causative agents of diarrhoeal diseases. These are transmitted mainly because of improper disposal of sewage and non-availability of safe water. It is, therefore, essential to have close coordination and cooperation between public health and sanitary engineering laboratories. Such cooperation can be obtained by appointing veterinarians and sanitary engineering personnel to the advisory committees of laboratory services. This allows communication between health laboratory services and veterinary and sanitary engineering services, and also allows these units to be integrated with health laboratories.

Industrialization and other advances have given rise to a very dangerous situation—*pollution* of the environment. This threatens the existence of life on earth.

3. Structure of National Health Laboratory Services

Planning

LABORATORY SERVICES should be an integral part of the national health service, and should be developed within its framework. The planning, organization and administration of national health laboratory services varies from country to country and depends on many factors which need to be carefully considered. It is not possible to give a single model of a laboratory service which can be adopted by all countries. Information on the above points will help in determining the needs and deciding on the type of health laboratory most suited to a particular country.

Health laboratory services include laboratories of both public health and clinical medicine. The work of public health laboratories is mainly community-oriented, whereas clinical/hospital laboratories are mainly concerned with diagnosis and treatment of diseases in individual patients. Before choosing a model for a health laboratory service it is necessary to study the various models already available.

Model 1

In this system, university/medical college/hospital laboratories, public health laboratories and laboratories manufacturing biological reagents

Each country has to plan and develop its own laboratory system according to its specific needs, its health and environmental policy and the existing infrastructure.

first developed as independent laboratories, but were later organized into two main services, namely hospital laboratory services and public health laboratory services.

Disadvantages

- Lack of coordination between important public health and clinical activities.
- Duplication of staff, equipment and overhead costs.
- Highly qualified staff with expertise is required in two places while there is scarcity of such technical expertise.
- There is no standardization of laboratory methods in the two systems.
- Monitoring of performance is difficult as it has to be carried out separately for both services.
- Failure to make the best use of resources.
- Sometimes there are dangerous gaps in activities, while some important activities may be completely missed.
- The national health authority has difficulty in gathering epidemiological information.

Model 2

In the second system, university/medical college/hospital laboratories developed first and public health functions were gradually added to them.

Disadvantages

- Extra staff, facilities and funds for the additional work are not adequately provided.
- Specially trained staff is not appointed for public health work.
- Often, laboratory work for clinical medicine takes priority while public health work is neglected or relegated to a position of secondary importance.

Model 3

The third model available is that of a single integrated health laboratory service. There are many advantages of having an integrated laboratory service carrying out work in both public health and clinical medicine. This system can be readily adopted in countries where there is either no health laboratory service or it exists only in a rudimentary form. When segregation between public health and clinical laboratories already exists in a country, coordination between both types of laboratories is essential.

If such an integrated laboratory

Factors to be considered while planning a health laboratory service

- The constitution of the country. In some countries health is a central subject, whereas in others, each state/province is autonomous in health matters. In the latter instance, appropriate legislation may have to be made in order to coordinate national and state health laboratory services.
- The size and population of the country.
- The demographic structure.
- The geographic and climatic conditions.
- The cultural background of the population.
- The socioeconomic status of the people.
- The common health problems of the country.
- The national health plan and programmes.
- The availability of finances.
- The educational facilities (technical and laboratory medicine) available in the country.
- The transport and communication facilities.
- The availability of common technological facilities, e.g. safe water, electricity and gas.
- The availability of expertise and facilities for repairing equipment.
- The availability of laboratory technical facilities, e.g. reagents, chemicals, media, antisera, laboratory glassware and equipment.
- The pre-existing health services and the laboratory services infrastructure.
- The different types of laboratories (other than health laboratories) that are already in existence.

Advantages of a single integrated laboratory service

- The arrangement is economical and there is no duplication of staff and equipment, while overhead costs are reduced through bulk purchase of supplies.
- Highly qualified staff is not distributed in two places and therefore there is better utilization of scarce technical manpower.
- There is uniformity and standardization of laboratory methods.
- Monitoring of performance becomes easier.
- Management is easier as there is a single administrative control.
- There is uniformity in training.
- Clinical and public health training of managers is possible.
- The credibility of laboratory results is higher.
- There is better control on quality of work.
- All laboratory needs of the country are covered without duplication and without leaving dangerous gaps in activities.
- The national health authority can readily obtain epidemiological information, while epidemiological surveillance and investigation are easier.

A single integrated laboratory service may be the answer to both public health and clinical laboratory work and may be readily adopted wherever there is no health laboratory service.

is to function properly and succeed in its aims and objectives, priorities for both public health and clinical laboratory work must be fixed and carried out without neglecting either type of work.

It may not be possible to have integrated laboratory services at all levels of service in every country. Laboratory services should be integrated at least at the periphery, while at intermediate, state/province and central levels laboratories may either be integrated or separate, depending on the circumstances and arrangements in the country. If the laboratories are separate at these latter levels, functional co-ordination, particularly with the peripheral laboratory services, must be achieved.

Laboratory services should be well structured and adequately funded and staffed. A single administrative control at national, provincial/state and lower levels is desirable. Laboratories should form a pyramidal structure, with the largest number located at the periphery and performing simple functions, followed by intermediate, state/provincial (in large countries) and finally central laboratories. Responsibilities at the central, intermediate and peripheral levels, as well as the relationship between these levels, should be defined.

Levels of Laboratory Services

Peripheral laboratory services

These services should be located at the level where people first come into contact with health care workers, and include services from village to rural hospital level. The structure of PHC services varies in the countries of the Region, although several echelons can be identified. These may not exist uniformly in all countries and their nomenclature is also variable.

The following description is merely for illustration:

a. Village level health care is given by a village health worker or health guide.
b. The sub-health centre, health post or clinic may serve several villages.
c. The health centre gives support services and serves some of the referrals from village and sub-health centre levels. The health centre serves several sub-health centres although there are large variations in the number of people covered by a health centre and in the staffing patterns.
d. The rural hospital or primary level hospital or community health centre constitutes the uppermost level of peripheral laboratory services.

In some countries, certain echelons might be combined (for example, b and c or c and d) or one or two echelons might be absent. In most countries, no laboratory services exist at the first two levels. In spite of the need for such services at these levels, it is technically and administratively too difficult to provide them. The only activities which should be carried out at these levels are the collection and dispatch of specimens and possibly the introduction of very simple tests such as urine analysis and haemoglobin estimation. Another important activity at these levels is to mobilize the help of the community in initiating investigations.

Peripheral laboratories should be located at levels c and d. These laboratories provide technical support for preventive, curative and promotive services for both the community and the individual. Adequate attention must be given to

the establishment of laboratories at the periphery by the health laboratory services. While the administrative responsibility of laboratories at peripheral levels falls upon the medical/health officer in charge of the peripheral health care facility, the technical supervision and guidance, quality control, proficiency testing, provision of reagents and equipment and training of technical staff should be the responsibility of the intermediate or central laboratory.

Intermediate laboratory services

In most countries, a district is the natural choice for the next level of laboratory service. Most district/regional headquarter hospitals do have a clinical laboratory which can be the focal point, networking laboratory services at the periphery with the state or central laboratory.

A network of laboratories must ensure coordination and cooperation between laboratories of different levels.

In some countries, there are public health laboratories at the district level which can provide similar networking between peripheral laboratory services and the central/state laboratory. Some countries establish integrated intermediate level laboratories which perform the functions of both public health and clinical medicine.

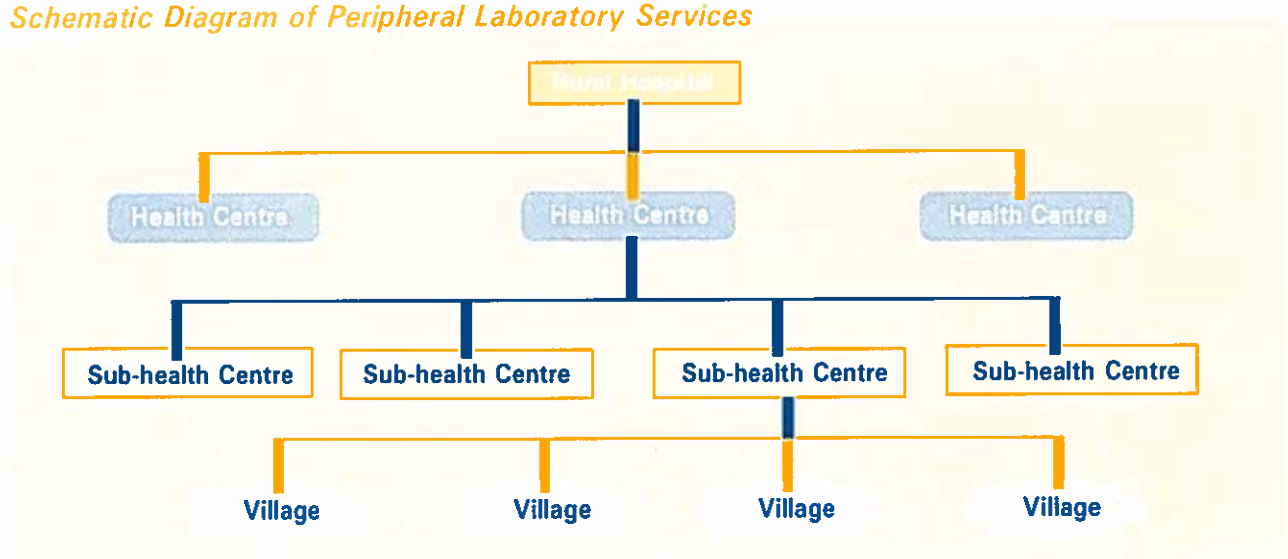
Since intermediate laboratories have important functions of providing support (both logistic and technical), supervision, guidance, quality control, proficiency testing, and selection and training of staff for peripheral laboratory services, a district level laboratory (intermediate laboratory) should be very well equipped, adequately funded and staffed and carefully planned.

It is also desirable to have a full-time epidemiologist at each intermediate level laboratory.

Central/state or provincial laboratory services

The central (or national) health laboratory is the principal laboratory of the country, and is technically responsible for the entire network of health laboratories. Its tasks are multiple and include both administrative and technical functions. In the early stages of the development of laboratory services, the central laboratory can be both the technical and administrative headquarters of the service. In most countries, some

Schematic Diagram of Peripheral Laboratory Services



type of central public health laboratory exists but a similar central laboratory for clinical medicine is lacking. The value of both types of central laboratory is undoubted. Each type provides guidance in methodology, develops training, and carries out referral work and quality assurance in specific fields.

As discussed above, it is advantageous to have a single integrated laboratory for both public health and clinical medicine work. In large countries with a number of states/provinces, each state may be autonomous in health matters and each state should have a state/provincial laboratory. These state laboratories have functions similar to those of the central laboratory, and carry out the full range of tests needed for the preventive, curative and promotive needs of the community and individuals. A full-time epidemiologist is required both for the central and for each of the state or provincial level laboratories.

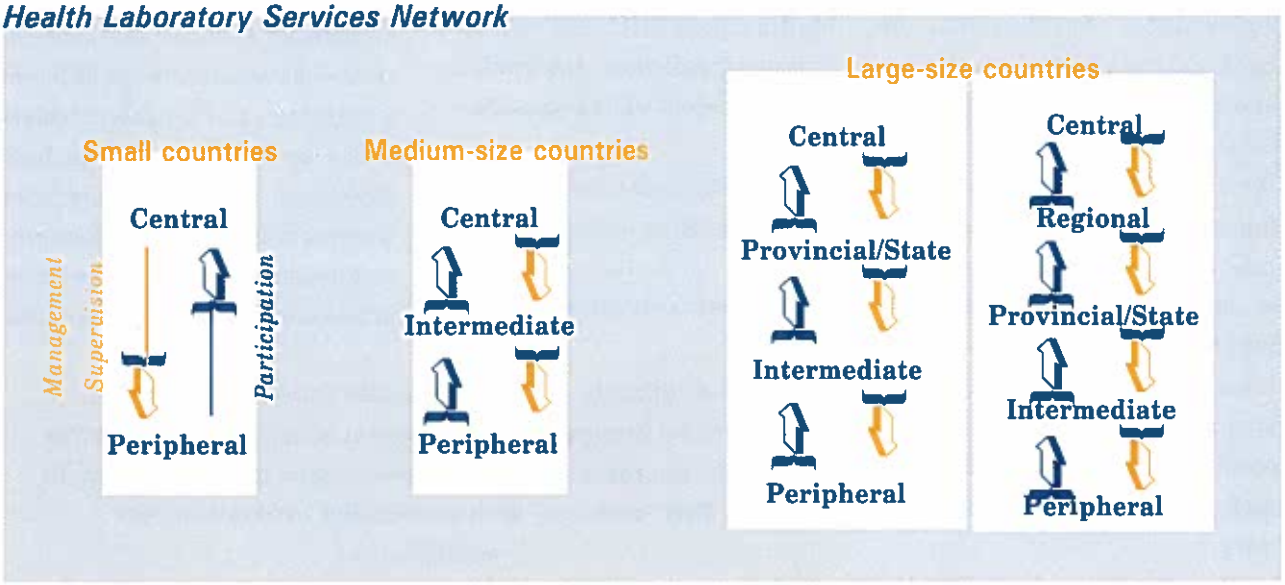
Efficient networking is one of the most important prerequisites for running a successful health laboratory service.

Networking and Linkages Between the Various Levels of Health Laboratories

The mere presence of adequately equipped and staffed laboratories at each level is not enough to achieve the goal of an efficient and properly managed health laboratory service unless very close coordination, cooperation and linkages exist between the different echelons of laboratory services.

Efficient networking is the most important prerequisite for running a successful health laboratory service. To ensure close coordination and cooperation between public health and clinical medicine work and also between the various levels of laboratory, it is necessary to change the attitude of laboratory personnel so that they develop a holistic approach to laboratory work. The networking should be very close so that the entire laboratory service runs in an integrated manner. Adequate staff, equipment, and, above all, funding for transport and communication between the different echelons of service must be provided to achieve this goal.

Health Laboratory Services Network



The presence of a full-time epidemiologist on the staff of the central, state/provincial laboratory (and, if possible, of intermediate laboratories) will ensure close cooperation and coordination between public health and clinical medicine laboratory work.

Very often, adequate funds are not provided for transport and communication, although these are absolutely essential for supervision, referral, reporting and personal discussion.

The central/state or provincial laboratory supervises and supports the work of intermediate laboratories, whereas intermediate laboratories supervise and support the work of peripheral laboratories. Referrals and laboratory data are sent from the peripheral to the intermediate to the state or provincial/central laboratory, while monthly/quarterly supplies, visits by supervisors, instructions on methodology, training programmes and quality assurance flow from the central/state laboratory to the intermediate to the peripheral laboratory. The presence of a full-time epidemiologist on the staff of the central state/provincial laboratory (and, if possible, of intermediate laboratories) will ensure close cooperation and coordination between public health and clinical medicine laboratory work.

Integration of Vertical Health Programmes with Laboratory Components of Other Services

A lot of effort and resources are wasted at the administrative and technical levels by creating and maintaining independent networks of laboratories such as those for vertical disease control programmes (malaria, tuberculosis, leprosy) and veterinary, water, food and environmental services, which are directly or indirectly involved in human health care. The ultimate goal of the health laboratory services should be integration of all essential activities related to health laboratories into comprehensive and multipurpose PHC services, with continuing evaluation and modification/adjustment when necessary.

There are many advantages of integrating these services and those of other laboratories, such as environmental services, with the basic infrastructure of the peripheral laboratory service. Such integration leads to better utilization of services, improved cost–effectiveness, and wider population and environment coverage. Also, it is rational, and the use of standard equipment and reagents throughout the peripheral network allows better servicing, maintenance and repair. Integration, therefore, leads to increased efficiency of PHC delivery and disease surveillance. These objectives can be achieved by proper training and motivation of laboratory staff at the periphery, close supervision, improved quality control, proper logistic support, supply of written guidelines on methodology and by personal discussion and demonstration during supervisory visits to peripheral laboratories.

If such integration is not possible in the immediate future, a mechanism should be developed for close cooperation between different programmes and peripheral laboratories by sharing work in both directions. This will require prior training of laboratory workers who may need to undertake work which is different from that in their own field.

The ultimate goal of the health laboratory services should be integration of all essential activities related to health laboratories into comprehensive and multipurpose PHC services, with continuing evaluation and modification.

4. Collaboration and Cooperation of the National Health Laboratory Services with Other Laboratories

Collaboration with University/Medical College/Reference Laboratories

UNIVERSITY/MEDICAL College laboratories are autonomous and under different administrative control. Teaching, training and research are the primary aims of such laboratories, while they also render laboratory services for the teaching hospitals attached to them. These laboratories should cooperate with the health laboratory service by providing reference and research facilities, consultancy services, and training of personnel. They may also be requested to send laboratory data to the health laboratory service so that complete epidemiological data for a country can be generated.

Reference laboratories specializing in a specific area of laboratory work may be either central or state laboratories or, depending on the interest and initiative of individual scientists, university/medical college/veterinary laboratories. Reference laboratories receive referrals in their specialized fields, render consultancy services, prepare and standardize diagnostic reagents, and carry out research and training in their specific areas. They also maintain close cooperation with international reference centres. A mechanism must be developed for continual functioning and sustainability of reference centres, whether they are in universities or are public health laboratories.

Cooperation with other Laboratories

Close cooperation and coordination must be developed between health laboratory and veterinary services. This may be achieved by having a veterinarian on the advisory committee of the health laboratory services, as well as attaching or even integrating some veterinary service laboratory units with health laboratories.

The health laboratory service should cooperate with sanitary engineering, industrial, agricultural, environmental control and medico-legal laboratories to achieve horizontal coordination with laboratories concerned with human health.

The health laboratory service should also coordinate and cooperate with other laboratories concerned with human health, such as sanitary engineering, industrial, agricultural, environmental control and medico-legal laboratories. Thus, horizontal coordination with all types of laboratories dealing with human health should be achieved.

Role of nongovernmental organizations

The contributions of non-governmental organizations in delivering essential clinical services to low-income households in the poorest countries has been remarkable. In certain countries of Africa, they provide more than one-third of all clinical care. In India and Indonesia, they supply more than 10 per cent of clinical services (World Development Report, 1993).

Role of private laboratories

Private laboratories are mostly commercial enterprises, except for the few private foundations which have other objectives such as service, research and training. They should be encouraged to participate in various quality assurance programmes and assist in laboratory diagnosis of common diseases.

Through their active involvement, they could assist locally in disease control and surveillance.

It is expected that health laboratory services will be self-sufficient for routine services but, at times, the help of private laboratories may be required. The professional competence of all laboratories, both public and private, should always be under close scrutiny.

International collaboration for a global network

The challenges faced by the world for early detection and control of epidemics can be met by strengthening the national infrastructure for routine surveillance of common diseases. A global network of WHO Collaborating Centres has been formed. The network remains alert and provides technical support to detect and effectively control epidemics of communicable diseases.

Since 1996, WHO is coordinating an international effort to detect and control communicable diseases through an efficient surveillance system and upgraded diagnostic capabilities.

There is no doubt that countries will continue to face outbreaks due to emerging and re-emerging infectious diseases. Especially in a poor health environment an unusual event may not be detected until it becomes a major threat to the population and cannot be contained with national resources. The existing public health laboratories are often poorly equipped or unable to diagnose common diseases. Thus the countries must develop a national infrastructure for surveillance systems and strengthen health laboratory services.

Since 1996, WHO is coordinating an international effort to detect and control communicable diseases through an efficient surveillance system and upgraded diagnostic capabilities. It works in close co-operation with local health authorities and international organizations. An improvement of public health infrastructure and enhancement of expertise and linkages are important components of this infrastructure.

National laboratories are being identified and would soon be linked electronically to facilitate rapid exchange and transmission of information.

5. Phased Development of the National Health Laboratory Services

ESTABLISHMENT OF a national health laboratory service may be a slow process in developing countries, extending over many years. Implementation can be carried out in a phased manner and completed in five to six years, perhaps starting with a few peripheral, intermediate and central laboratories. Allotment of funds, manpower requirements and production, suitable buildings for network laboratories and procurement of equipment and supplies should be carefully planned. The number of technical personnel required (both medical and paramedical) should be calculated on the basis of staffing norms (mentioned elsewhere), and recruitment of the necessary staff should commence within the country if facilities exist, or in other countries of the Region. In some countries, foreign nationals may be taken initially on contract for a limited period of time, while consultants from abroad may be invited to advise in specific areas.

To attract talent to the health laboratory service, it is essential to develop a generous pay scale with allowances and promotional avenues within the laboratory set-up.

6. Research

RESEARCH STIMULATES interest and satisfies the intellectual curiosity of laboratory personnel, and helps to attract and retain competent laboratory workers. Research projects should be undertaken according to the needs of the country. Research requires well-trained and experienced personnel. It should largely be carried out at central and state/provincial laboratories, and involve intermediate and peripheral laboratories in the collection of samples and epidemiological data.

Research projects should be undertaken according to the needs of the country.

However, the staff at intermediate and peripheral laboratories should be encouraged to actively participate in the enumeration and implementation of operational research and simple epidemiological research. This will generate interest and provide intellectual satisfaction to staff at the periphery.

7. Range of Tests in the National Health Laboratory Services

RECENT HEALTH CARE trends should be taken into account while reorganizing health laboratory services. These include the increasing emphasis on tests that help in prevention, rapid diagnostic tests that permit earlier intervention, decentralization of certain testing procedures to the PHC level, regionalization of referral centres, clinical or public health relevance of specific tests, cost–effectiveness, modern methods of management, choice of tests for different echelons of laboratory according to the actual needs and priorities of the country concerned, available resources, rationality and changes in morbidity.

In view of the multidimensional developments and trends affecting laboratory services, the central laboratory should develop a policy regarding the range of tests and the technologies to be used.

Laboratory tests should be assessed comprehensively before they are introduced into the network and should be withdrawn if they become obsolete. This assessment should take into consideration technical efficiency, cost, logistics, maintenance of equipment and clinical/public health relevance of each test.

In most countries of the Region, diarrhoeal and other infectious diseases and microbiological control of drinking water and food need to be accorded top priority.

Tests of Public Health Relevance

Priorities must be allotted to tests with relevance to public health depending on the areas of concern. These include control of infectious diseases (according to the epidemiological patterns in the country), control of water (drinking water has priority over bathing and surface water or sewage), control of food (microbiological testing has priority over the detection of pesticide or hormone residues as the latter require considerable laboratory infrastructure and highly qualified staff—certainly not a priority for developing countries), and control of the environment (e.g. air pollution, dangerous chemicals, toxic wastes and radioactivity).

Factors to be considered for tests recommended at all levels

- Changes in technology which make determinations more practicable and/or affordable (e.g. colorimetric procedures for sodium or potassium).
- Changes in technology which affect the particular method recommended for a test (e.g. glucose oxidase procedures for glucose).
- Which procedures need to be replaced by alternative or better ones.
- Changes in technology which serve more than one discipline, e.g. the use of similar immunological techniques for hormone assays (clinical chemistry) and for diagnosis of infectious diseases (microbiology).
- Coordination of activities in different disciplines (e.g. a clinical chemistry technique, such as estimation of serum/plasma iron, may make similar haematology investigations unnecessary).
- Changes in treatment available at the PHC and intermediate levels.
- The introduction of services at one level which result in reduced demand at other levels (e.g. the introduction of the test for fructosamine or haemoglobin A1 [HbA1] at an intermediate laboratory could reduce the need for glucose determination at the PHC level for diabetes control).

The choice of priorities depends mainly on epidemiological data. In most countries of the Region, diarrhoeal and other infectious diseases and microbiological control of drinking water and food deserve high priority.

Serological tests are useful in epidemiological surveys of many communicable diseases, and enable the immunization status of the population to be assessed through the measurement of antibody levels. Due to the recent pandemic of HIV infection, sero-surveillance and sentinel surveillance studies have become a top priority in most countries.

Tests of Clinical Relevance

Priority should be given to common diseases. It must also be considered whether priority should be given to acute rather than chronic diseases and to curable rather than non-curable diseases. For example, in developing countries, the maximum mortality and morbidity are due to communicable diseases which are often acute in their clinical course; whereas chronic diseases, such as diabetes, hypertension and coronary artery disease, are of concern in developed countries. Therefore, the choice of laboratory testing facilities will depend on the prevailing disease pattern in the country. Priorities must also be fixed according to the demand from physicians and the public.

Appropriate Technology

Appropriate technology is defined as 'adaptation of knowledge and skills which are scientifically sound and acceptable to those who apply them and to those for whom they are used, to local circumstances and conditions'.

Developments in biotechnology, introduction of advanced materials, microanalytical methods, computerization and the application of non-invasive methods, have led to

the introduction of a wide range of kits and rapid automated methods. These have also led to a reduction in size and increase in portability of laboratory equipment, thus permitting tests to be carried out not only at the periphery but in very remote areas as well.

An example of an appropriately tailored equipment for the periphery is the portable MacArthur microscope. Tests which are reliable, cheap, and easily and rapidly performed, are needed particularly in peripheral laboratories.

Introducing Newer Technologies

Rapid diagnostic tests

Conventional laboratory tests, though sensitive and specific, are often time-consuming, difficult to perform and need many reagents and equipment. Therefore, there is a need to introduce tests that are rapid, simple, reliable, reproducible, and cost–effective. A wide range of such tests is available for infectious diseases—bacterial, viral, mycotic and parasitic (both for antigen and antibody detection), clinical chemistry and clinical pathology. Many of these tests are slide agglutination tests such as coated latex or gelatin particles and coagglutination, while others include the dipstick, dot blot ELISA, liposome immunoassay, and culture screening tests for urine, etc. These tests are discussed in the sections on appropriate technology.

The introduction of rapid tests in peripheral laboratories will allow prompt patient management and better disease surveillance, which will be a great support for the PHC. The central laboratory should introduce the tests after careful assessment. Apart from technical evaluation (reliability, sensitivity, specificity, reproducibility, technical simplicity), clinical utility and the cost–benefit ratio, shelf-life and availability of kits should be assessed. The central laboratory should prepare a list of tests which are appropriate for peripheral laboratory services and decide on the type of test to be introduced at the periphery. The central laboratory should procure and supply kits, reagents and other materials for carrying out such tests. They should arrange training courses before introducing the tests at lower levels. Initially, such kits may have to be imported from developed countries, although the possibility of manufacturing the kits and reagents in the country, or at least in some countries of the South-East Asia Region, must be considered.

Automation may appear very appropriate for countries of the Region. However, the equipment is expensive and its maintenance and repair is complex.

Automation

Automation is a recent development in laboratory technology that is widely used in clinical chemistry, haematology and now, also in microbiology. Automation may appear appropriate for those countries of the Region which lack trained technical personnel. However, the equipment is expensive and has to be imported, and its maintenance and repair is complex. Also, the reagents used are costly and imported; recurring expenditure is thus high. Automation is cost–effective only if 50–100 samples are processed at any one time. This makes the technology unsuitable for peripheral laboratories.

In many developing countries, locally-made semi-automated blood cell counters are now available, which are not too expensive. These replace counting chambers for haematological procedures and can function with locally-made reagents. These could be recommended for intermediate laboratories.

If resources permit, use of automated instruments can provide reliable RBC and WBC counts even when the number of samples to be tested is small.

First it should be decided whether automation is required in the Region, and, if so, in which laboratories (which levels) and in which disciplines (clinical chemistry, haematology, microbiology). In many big hospitals and university/medical college laboratories of the Region, automation has already been introduced for clinical chemistry and haematology. In the health laboratory services, such technology should be initially introduced in the central and state/provincial laboratories. The technology will never be cost–effective for laboratories at lower levels.

In the health laboratory service, automation should be initially introduced in central and state/ provincial laboratories. The technology will never be cost–effective for laboratories at lower levels.

Advanced technology

Recent developments in molecular biology and immunology have resulted in a large number of tests that can detect minute quantities of specific genetic material, enzymes, peptides and proteins of infectious agents. Such tests include: polymerase chain reaction (PCR); gene hybridization—DNA, RNA probes; electrophoresis—peptide, protein, reverse field; immunoblotting; immunofluorescence and enzyme immunoassays. Molecular techniques to type microorganisms for epidemiological studies include: plasmid profiles (fingerprinting); restriction endonuclease of plasmid (REAP) or genome by restriction fragment length polymorphism (RFLP); pulse field gel electrophoresis; ribosomal RNA (rRNA) typing (ribotyping); pyrolysis—mass spectrophotometry; enzyme profiling (multilocus enzyme typing) and electrophoretic karyotyping. PCR is gaining importance as a diagnostic tool and though the technology is costly and requires sophisticated equipment at present, reagents and containment facilities as well as highly trained technologists and facilities should be available in central laboratories.

8. Peripheral Laboratory Services

Echelons of Peripheral Laboratory Services

The village/sub-health centre

AT THIS LEVEL, laboratory work is carried out by health workers and not technicians. The function of the laboratory at this level is mainly limited to the collection of samples, which are referred to a health centre or rural hospital, and to conduct simple tests such as urine analysis and haemoglobin estimation. There are large variations in the nomenclature, staffing patterns and populations covered by sub-health centres, and the range of tests should be decided on the basis of the manpower and resources available. Short-duration training programmes and periodic refresher training in relevant laboratory techniques are required.

The health centre/rural hospital

At this level, there are large variations between countries in the nomenclature, staffing patterns and populations covered. The range of tests should be decided according to the needs, availability of resources, staffing patterns and policies of the health service.

These laboratories help in the curative, preventive and promotive services, and provide referral services. These centres also provide ante- and postnatal care and family planning services, diagnose common diseases and carry out public health activities.

Functions of Peripheral Laboratories

Laboratories at this level should be integrated and should carry out both clinical and public health activities at a basic level. These laboratories can help with common diseases. These include parasitic (malaria, filariasis, vaginal trichomoniasis, amoebiasis and other intestinal parasites), bacterial (tuberculosis, leprosy, gonorrhoea, meningitis, skin infections with pyogenic organisms), fungal (candidiasis, superficial mycoses) and noncommunicable diseases (anaemia, diabetes, eclampsia). For viral infections, only sample collection and dispatch to the intermediate/central laboratories is possible, unless rapid diagnostic kits are introduced.

Facilities are limited at this level and only the simplest tests are performed. The range of tests to be carried out should be decided by each country, and those mentioned on page 20 are only suggestions. Tests may be added or deleted according to local arrangements and circumstances.

Diagnosis

Diagnostic procedures available at this level depend on the resources, manpower and policies of the health service and on the prevalent communicable and noncommunicable diseases. Need-based appropriate technology, which has been comprehensively evaluated by the central laboratory, should be used wherever possible (see Annex 1 for full range of tests).

Peripheral laboratories should be integrated so that they can carry out both clinical and public health activities at a very basic level.

Referral

Referral from this level to higher-level laboratories should include:

- Dispatch of faecal samples for culture, in suitable transport medium in cases of cholera, dysentery and diarrhoea.
- Serum/blood samples for culture and serology.
- Water samples for analysis and culture.
- Dispatch of appropriate samples during outbreaks. Technicians at the periphery must be trained to respond rapidly and appropriately in case of an outbreak.

All the above procedures require adequate training and experience in the standard procedures for collection, storage and transport of samples.

Tests

Tests which are reliable, sensitive, specific, cost–effective and easily as well as rapidly performed are needed in peripheral laboratories to support the PHC. Newer techniques, such as latex agglutination, coagglutination, and dipstick ELISA, can be very useful. Such tests have to be evaluated and introduced by the central laboratory.

Surveys

Laboratories at this level should participate in surveys and epidemiological studies organized by intermediate, state/provincial and central laboratories.

Record-keeping

Record-keeping is essential for the efficient functioning of laboratory services. Registers must be maintained properly, giving dates, name, age and sex of patients, clinical diagnosis and results of tests. These registers will generate laboratory-based surveillance data in peripheral laboratories.

Quality assurance

A suitable quality assurance programme must be established.

Communication

There should be proper communication between peripheral and higher level laboratories regarding data collection, supervision and quality assurance. Laboratory data

Basic microscopic examinations

- Faeces for exudate (amoebic/bacillary dysentery), trophozoites, ova and cysts.
- Vaginal swabs: wet preparations for *Trichomonas* and Gram-stained smears for candidiasis.
- Urethral discharge: Gram-stained smears for gonococci.
- Sputum: Ziehl–Neelsen stained smears for acid-fast bacilli (AFB).
- Skin: Ziehl–Neelsen stained smears for AFB and direct wet preparations for fungi.
- Ziehl–Neelsen stained nasal smears for AFB.
- Blood films for malarial parasites and microfilaria (Romanowsky stain).
- Urine deposits for cells, casts and parasites.
- Pus and exudate: Gram-stained smears for bacteria.
- Cerebrospinal fluid (CSF): Gram-stained smears for bacteria, wet preparations for parasites and cell counts in counting chambers.
- Semen examination.

Clinical chemistry examinations

- Urine examination for glucose, protein, blood, ketones, bile pigments and salts, nitrites and pregnancy tests.
- Faeces for occult blood.

Haematology examinations

- Haemoglobin estimation.
- Total leucocyte count (TLC).
- Differential leucocyte count (DLC).
- Erythrocyte sedimentation rate (ESR).
- Packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC).
- Bleeding and clotting time.
- Peripheral blood smears for cell morphology.

Additional examinations

- Water (coliform count, preferably by kit).
- Common salt (analysis for iodine content).

generated at the peripheral level should be appropriately used for effective health care at this level. Such laboratories at the peripheral level provide greater motivation for physicians and health care workers (HCWs) at this level, improve patient perception of the care received as well as patient confidence in the capabilities of the peripheral health services. Thus, more people will be encouraged to utilize services at the periphery.

Laboratories at the peripheral level provide great motivation for physicians and health care workers at this level, improve patient perception of the care received as well as patient confidence in the capabilities of the peripheral health services.

Staff

The staff in peripheral laboratories should include one technician and one laboratory assistant/attendant.

Space

Peripheral laboratories should include one laboratory-cum-office/record room (5 m × 3 m) and one storeroom combined with other services (5 m × 3 m).

Equipment/Supplies

Necessary equipment and supplies include:

- good equipment—microscopes, centrifuges, haemoglobinometers, transport media, glassware, sterile swabs;
- reagents
  - for staining (e.g. Gram, Ziehl–Neelsen, Romanowsky stains)
  - for chemical examination of urine
  - for iodine analysis of common salt, and
  - kits for rapid diagnostic tests;
- sterilized syringes and needles
- collection bottles for blood/serum and water analysis.

Other Facilities

Other necessary facilities include supply of safe water, a reliable source of energy (battery, electricity, solar or kerosene) and sterilization facilities. There must also be transport and communication facilities between the peripheral laboratory services and intermediate laboratories for referral of samples and patients, procurement of supplies and personal discussion.

9. The Intermediate Laboratory

THE INTERMEDIATE laboratory is located at the district/regional headquarters, while the clinical laboratory is located in the district/regional hospital. As discussed earlier, the public health laboratory can either be integrated with the clinical laboratory or can exist as a separate laboratory. The size of the intermediate laboratory will vary from country to country and within a country, depending on the size of the population served.

All peripheral laboratory services of a particular district/region will be attached to the intermediate laboratory of that region.

The activities of intermediate laboratories depend on the health needs, population served, manpower and resources available, and demands on the service. The range of tests to be conducted should be decided by the central laboratory of each country after discussion with the staff of laboratories at other levels.

Basic facilities to be provided by each section of the laboratory

Microbiology and Parasitology

- Basic microscopical work for evidence of inflammation, exudate, bacteria, fungi, protozoa, helminths.
- Darkfield microscopy for *T. pallidum*.
- Culture and antibiotic sensitivity testing of clinical specimens such as faeces, urine, blood, pus, CSF, throat swabs, aspirated fluids, and urethral discharge.
- Bacteriological examination of water (all important bacterial isolates should be referred to state/provincial or central laboratories for full identification, characterization and typing).
- Dispatch of samples to state or central laboratories for virus detection (e.g. rotavirus, encephalitis) and serology.

Serology

- The venereal disease research laboratory (VDRL) test.
- Agglutination tests.
- Antistreptolysin O test.
- Immunological techniques for pregnancy.
- Viral serology (as and when directed by the health authorities/central laboratory).
- Screening for human immunodeficiency virus (HIV) and hepatitis B surface antigen (HBsAg). The screening tests selected should be rapid, simple, sensitive, reliable and cheap.

Clinical biochemistry

- Urine examination (glucose, protein, ketones, bile salts/pigments, urobilinogen, pregnancy testing and microscopic examination of deposits).
- Blood examination (glucose, urea, bilirubin, protein, alkaline phosphatase, and serum glutamate oxaloacetate transaminase [SGOT]).
- CSF examination (glucose, protein).
- Faeces examination (occult blood).

Basic facilities . . . continued on facing page

Functions of the Intermediate Laboratory

The intermediate laboratory assists in the diagnosis and treatment of individual patients at its attached hospital, and is also used as a public health laboratory for epidemiological surveillance and control of diseases within the community. The work undertaken by the intermediate laboratory is of a relatively simple nature but the range of tests conducted is much wider than at the peripheral level. More complex tests are referred to the state/provincial or central laboratories. The intermediate laboratory trains technicians and health workers to carry out laboratory tests for the peripheral laboratory services, and provides technical support to peripheral laboratories through supervision, personal discussion, quality control standardization of tests, instrument maintenance and in-service training. It also helps in developing criteria, standards and guidelines on the appropriateness, relevance, and cost–effectiveness of tests, in coordination with other intermedi-

Blood transfusion

Blood should be supplied from a blood transfusion centre or from a hospital blood bank after appropriate testing. Voluntary blood donation should be encouraged.

- Selection of donors on the basis of health questionnaires, blood pressure and weight determinations, haemoglobin estimations and screening for HIV-1 (HIV-2 also if considered to be prevalent), HBsAg and syphilis (VDRL, Rapid plasma reagin [RPR]) as well as other infections as decided by the national policy.
- Compatibility testing for ABO blood groups and Rhesus factor D (RhD) cell groupings with cross-matching at room temperature (saline tube test) followed by immediate centrifugation and anti-human globulin test.

Haematology

- Haemoglobin estimation.
- TLC and DLC.
- Peripheral blood smears.
- RBC count and PCV, erythrocyte sedimentation rate determination (ESR).
- Eosinophil, platelet and reticulocyte counts.
- Bone marrow examination.
- Bleeding and clotting time estimation.
- Prothrombin time estimation.
- Lupus erythematosus (LE) cell preparation.
- Blood grouping [ABO/Rh(D)].

Cytology

- Early detection of cancer.
- Cervical specimen examination.
- Fine needle aspiration cytology (FNAC).

Histopathology

- Examination of biopsy and organ material removed during operations.
- Postmortem examinations.

ate, state and central laboratories. Above all, the intermediate laboratory serves as a link between the state/provincial/central laboratories and peripheral laboratories in terms of:

- purchase of equipment;
- distribution of reagents/media/laboratory manuals and other supplies;
- data and information flow (as well as their collection, storage and, at times, analysis);
- effective monitoring; and
- quality assurance for lower level laboratories.

Tests

The intermediate laboratory should have sections for microbiology, serology, parasitology, clinical biochemistry, haematology, cytology and, if possible, histopathology (see Annex 1 for full range of tests).

Supplies

The intermediate laboratory should supply reagents, glassware, transport media, diagnostic kits, etc. to the peripheral laboratories. This

Staff

Intermediate laboratory staff should include:

- Qualified pathologist/microbiologist (Doctor of Medicine/Diploma in Clinical Pathology) — One
- Technicians (Diploma in medical laboratory technology [DMLT]) — Three
- Laboratory assistants (DMLT) — Two
- Laboratory attendants — Two
- Cleaner — One
- Clerk-cum-storekeeper — One

If it is not possible to have a full-time epidemiologist, the help of an epidemiologist should be available.

Space

One room each of stated size should be provided for:

- Microbiology/serology laboratory (9 m x 5 m)
- Clinical biochemistry, haematology, cytology, histopathology laboratory (9 m x 5 m)
- Sterilization and media preparation laboratory (6 m x 5 m)
- Storeroom (3 m x 5 m)
- Office (3 m x 5 m)

Equipment

The intermediate laboratory must have the following equipment:

Binocular microscope with in-built light	Two
Darkfield microscope	One
Inoculating chamber	Two
Centrifuge	Two
Autoclave (downward displacement type)	Two
Incubator	Two
Hot air oven	One
Water bath	Two
VDRL shaker	One
Photometer	One
WHO Haemoglobin colour scale*	Few
Blood cell counter	One
Microhaematocrit centrifuge	One
Haemoglobinometer	Several
Haemocytometer	Several
Westergren tube and stand	Several
Refrigerator	One
Balances	Two
pH meter	One
Inspissator	One
Deionizer/ distilled water apparatus	One
Microtome	One
Histokinette	One

(*especially for domiciliary visits, and antenatal clinics)

function may vary from country to country depending on the arrangements in each country.

Laboratory animals

The intermediate laboratory should have facilities for laboratory animals, e.g. sheep, guinea pigs and rabbits for preparation of culture media, isolation of pathogens and serology, etc. Laboratory animals should be obtained from the state/provincial or central laboratory.

Record-keeping

Accurate and up-to-date laboratory records should be kept.

Information

Data generated from the intermediate laboratory should be sent to the state/provincial/central laboratory and to the directorate of health services. Intermediate laboratories should also help to collect data from peripheral laboratories, as well as store data and, at times, help in analysis.

Surveys

Intermediate laboratories should participate in surveys conducted by the state/provincial/central laboratory.

Quality assurance

Intermediate laboratories should implement quality assurance programmes by integrating internal quality control measures with their routine work and participating in appropriate external quality assessment schemes. They should also supervise quality assurance activities at lower level laboratories.

Visits to peripheral laboratories

Suitable staff members from intermediate laboratories should visit peripheral laboratories at frequent intervals for supervision, personal discussion, in-service training, quality control and guidance.

10. The Central/State or Provincial Laboratory

THE CENTRAL AND STATE (or provincial) laboratories are considered together because in large countries, with many states (provinces) which have autonomy in health matters, every state should have a central laboratory known as the state or provincial laboratory. In addition, the central government should have a national central laboratory. The state laboratories have similar functions to those of the central laboratory, and carry out the full range of tests required for the curative, preventive and promotive health needs of the community. The state laboratory also carries out all other administrative and technical functions of the state (province) health laboratory service, as does the central laboratory.

To effectively coordinate the work of all state (provincial) and central laboratories, there should be a health laboratory service board that implements national health policies and programmes.

Functions of the Central/ State or Provincial Laboratory

The central laboratory is the principal laboratory of the country, and is technically and administratively responsible for the entire network of health service laboratories. It is also responsible for the planning, organization and supervision of the whole laboratory service.

Administratively, the central laboratory coordinates the activities of the laboratory network, issues administrative instructions and requests, receives periodical reports from the laboratory network for evaluation, stores and analyses useful data (principally epidemiological) and represents the laboratory network in the concerned Ministry and Directorate of Health Services to ensure that the health laboratory service functions and develops in accordance with the national health policy.

The central laboratory also advises the Ministry and Health Directorate of the progress, needs and aspirations of the laboratory service and, above all, plans and provides full justification for the allocation of funds.

Technically, the central laboratory must be capable of performing the full range of tests and activities in public health and clinical medicine and must therefore have all the necessary laboratory and technical departments. Considering the high prevalence of communicable diseases in countries of the Region, the microbiology and serology sections should be well developed.

It is preferable to have a full-time epidemiologist in the central laboratory. This would permit necessary and constant cooperation between epidemiological and laboratory staff, and encourage a public health outlook in the laboratory service while directing the attention of the service to the field (community) as well as to the hospital (patient). The laboratory service should benefit greatly from sophisticated and modern epidemiology in assessing the public health priority of various diseases and in formulating the vaccination policy when evaluating vaccination and control programmes and designing and carrying out surveillance activities such as sentinel surveillance of HIV infection.

The central laboratory must carry out the full range of tests required for the curative, preventive and promotive health needs of the community.

The work of the central laboratory should be mainly directed towards national and community needs and should be planned according to the policies of the various ministries concerned with human health (such as those of disease surveillance, epidemiological investigations, evaluation of control programmes, water and food sanitation, maternal and child health, and school health).

From the clinical laboratory point of view, the central laboratory should offer a complete range of tests for samples referred from lower level laboratories for confirmation and detailed analysis during epidemiological surveys and outbreaks. The central laboratory does not generally carry out routine diagnosis except for highly specialized tests, e.g. PCR, radioassays.

A documentation service, supported by an information system capable of processing and storing the data collected and with access to a comprehensive and up-to-date library, is of prime importance. The central laboratory should prepare and distribute periodical (monthly/quarterly and annual) reports, containing statistics on the prevalence of diseases and recommendations for treatment and control, to all lower level laboratories and the national/state health authorities.

Facilities

The central laboratory should have facilities for various branches of laboratory medicine (see Annex 1 for the list of tests).

Bacteriology/Serology/Virology/Mycology/Parasitology

- A complete range of diagnostic activities. Facilities for characterization (including their antibiotic sensitivities) of all pathogens.
- Typing of pathogens for epidemiological studies, such as phage typing of *Staphylococcus aureus* and *Salmonella typhi;* serotyping of *Salmonella, Shigella, Escherichia coli* and meningococci; *Streptococcus pyogenes* and differentiation of enteropathogenic, enterotoxigenic, enteroinvasive, enterohaemorrhagic and enteroadherent strains of *Escherichia coli*.
- Investigation of outbreaks of gastroenteritis, acute respiratory infections, haemorrhagic fever, unexplained fevers, etc.
- Culture facilities for anaerobic bacteria, *Mycobacteria, Brucella, Chlamydia,* etc.
- Detection of *Clostridium difficile* toxin.
- A plasmid reference laboratory to study the epidemiology of drug resistance. Monitor drug sensitivity of enteric pathogens, gonococci, etc. and formulate therapeutic guidelines.
- Bacteriological examination of water, milk, dairy products and foods.
- Facilities for antistreptolysin O (ASLO), anti-deoxyribonuclease B (ADNase B), and C-reactive protein (CRP) for acute rheumatic fever and rheumatic heart disease.
- Diagnosis of syphilis, with facilities for *Treponema pallidum* haemadsorption (TPHA) and fluorescent treponemal antibody-absorbed (FTA-Abs) tests besides VDRL.
- Isolation and identification facilities for important viral agents (Japanese encephalitis [JE], polio, measles, rotavirus, herpes simplex, rabies, respiratory syncitial virus [RSV], etc.).
- Serological tests for HIV, viral hepatitis, JE, dengue, measles, and rubella.

Facilities *(Continued)*

- Special staining facilities for *Cryptosporidium*.
- Serology of parasitic organisms (*Entamoeba histolytica, Plasmodium falciparum, Toxoplasma*).
- *In vitro* testing of drug resistance of *Plasmodium falciparum*.
- Advise on vector control and bioenvironmental interventions.

Clinical Chemistry

In addition to the clinical chemistry tests performed at the intermediate laboratory level, the central laboratory should have the facilities and competence to carry out the complete range of tests mentioned in Annex 1. The central laboratory should organize quality assessment for all laboratories. Automated or semi-automated equipment would be useful at the central laboratory level for at least some investigations.

Haematology

In addition to the haematological tests carried out at the intermediate laboratory level, the more specialized tests mentioned in Annex 1 should be carried out. Automated or semi-automated equipment will probably be useful at this level. The central laboratory should organize quality assessment and standardization with the provision of reference preparations for the laboratories in the service.

The central laboratory concerned with transfusion services should be included in the national blood transfusion centre.

Histopathology and Cytology

- All facilities for early detection of cancer (FNAC, etc.).
- Histopathological examination of biopsies and organs removed during operations.
- Special staining facilities.
- Histochemistry facilities.
- Postmortem facilities.

Facilities

The central laboratory should have facilities for various branches of laboratory medicine (see Annex 1 for the list of tests).

Processing of referral samples

The central laboratory should process specimens received from intermediate/peripheral laboratories and confirm test results as well as carry out specialized tests on strains of microorganisms for further characterization.

Water analysis

Water analysis should be carried out for both chemical and bacteriological assurance. Similarly, milk, dairy products and foods should be tested for bacteria and toxic health markers (organophosphate pesticides, mycotoxins).

Drug analysis

Drug analysis and nutritional, toxicological and environmental pollution studies should be conducted.

Epidemiological surveys

This is an important function of the central laboratory.

Investigation of outbreaks

It is the responsibility of the central laboratory to investigate outbreaks of diseases in the country and advise on control measures.

Animal house

There should be up-to-date facilities for laboratory animals that allow breeding of pathogen-free, good-quality animals, as well as their supply to intermediate level laboratories.

Collection of data

The central laboratory should collect laboratory data from lower level laboratories, and process and prepare statistical data on the prevalence of various diseases. Statistics should be distributed periodically (quarterly, annually) and reports concerning the prevalence of diseases, with recommendations for their treatment and control, should be sent to all laboratories in the network and to the national/state health authorities.

Training courses

The central laboratory is responsible for developing and organizing courses for training technical personnel for the service. The staff of universities and intermediate laboratories may also be involved in this programme.

The central laboratory should also plan and organize training courses for paramedical staff (multipurpose workers, etc.), which should take place mostly at the intermediate laboratory level. Also, continuing education and refresher courses for all laboratory staff should be organized and laboratory manuals prepared for lower level laboratories.

The central laboratory should provide guidelines for record-keeping; formulate policies for purchasing; organize quality assurance programmes; and establish a biosafety policy.

Record-keeping

Record-keeping should be efficient and complete, and use of computers must be a priority. The central laboratory should provide guidelines to the whole laboratory network on the preparation of reports, reporting of test results and record-keeping.

Purchasing policy

The central laboratory should formulate policies for the purchase and supply of equipment, media, chemicals, reagents, etc. to laboratories in the network, while standardizing specifications and make. Whether purchasing is centralized or decentralized will depend on the arrangements in a particular country and will differ from country to country. The central laboratory should train staff in the care and maintenance of equipment and should provide guidelines for the standardization and selection of equipment and laboratory techniques.

Supervision of intermediate laboratories

The central laboratory should supervise intermediate laboratories and ensure that there is adequate communication within the different echelons of the laboratory network.

Research

The central laboratory should undertake research projects to resolve the public health problems of the country.

Quality assurance

The central laboratory should organize a quality assurance programme by initially arranging a workshop for laboratory personnel of lower level laboratories. This should be followed by visits and sending 'unknown' cultures, specimens, etc. to them. It should participate in international quality assessment programmes and external quality assessment schemes (EQAs)—both national and international.

Guidelines for biosafety

The central laboratory should prepare comprehensive guidelines for laboratory safety procedures. It is responsible for establishing a safety policy for all employees, and for implementing it in the entire network. It should monitor this implementation and ensure that the safety management system works at all levels.

Collaboration

The central laboratory should collaborate with international reference centres and centres of excellence in other countries of the Region.

Rapid diagnostic tests

The central laboratory should encourage the introduction of rapid diagnostic tests in lower level laboratories, depending on the availability of appropriate technology. It should prepare a list of diagnostic procedures appropriate for peripheral laboratory services, and the types of tests to be introduced should be decided only after careful assessment. The central laboratory should also procure and supply kits, reagents and other materials for carrying out such tests. It should organize training courses before introducing the tests at lower echelons of the laboratory network. The possibility of manufacturing the kits and reagents locally, in a phased manner, may be considered in the long run, although initially they can be obtained from outside the country.

Guidelines for staff requirements

Heads of laboratory divisions	One for each division
Laboratory specialists	Two to three in each division depending on requirement
Epidemiologist	One
Chief technician	One in each division
Technicians (MLT)	Several
Laboratory assistants (MLT)	Several
Veterinary officer for animal house	One
Animal house technician	One
Animal attendants	Several
Chemist (MSc or PhD)	One or two for water, drug and food analysis, toxicological studies and studies on environmental pollution
Computer professional	One
Cleaner	Several
Storekeeper	One
Clerical staff/stenographer	Several
Drivers	Depending on number of vehicles

Staff

The director of the central laboratory should be a specialist (MD) in a branch of laboratory medicine, with at least 15 years experience in clinical and/or public health laboratory work. If a fully qualified and experienced person is not available in the country, then initially a UN agency or a neighbouring country may help in recruiting a suitable person on a contractual basis.

The central laboratory should have several divisions, each dealing with a particular group or laboratory discipline. The number of staff will depend on the workload and needs of the laboratory, and will have to be worked out by each country.

Space

The central laboratory should have adequately sized laboratories for each section. Senior laboratory specialists should have laboratory and office facilities. There should also be a lecture theatre, a practical laboratory for trainees, seminar

rooms and rooms for stenographers, stores, records and offices. The animal house should have facilities for conducting operations and animal breeding, and adequate space to keep inoculated and uninoculated animals separately. The laboratory should also have a library with reading rooms. The exact size of the central laboratory complex will depend on the workload and requirements of the service as well as on the various constraints.

Equipment

In addition to the equipment listed for intermediate laboratories, the central laboratory should have the equipment listed in the box.

Additional equipment required in the central laboratory

- a spectrophotometer and a flame photometer
- millipore filters
- a laminar flow cabinet and biosafety cabinets
- anaerobic jars and an anaerobic chamber/incubator
- a BOD incubator
- a fluorescence microscope, an electron microscope
- apparatus for electrophoresis
- the full range of chromatographic apparatus including that for gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC)
- automated and semi-automated equipment for haematology and clinical chemistry
- an ELISA reader
- a freeze drying machine, deep freezes
- a refrigerated centrifuge, an ultra centrifuge
- a microtitre system
- a coulter counter, etc.

11. Successful Operation of Health Laboratory Services

A GOOD MANAGEMENT system is essential for the efficient functioning and full utilization of the health laboratory services. Proper attention must be paid to many points for optimal functioning of the laboratory service.

Coordination with Health Programmes

The laboratory service can contribute greatly to improving the delivery of health care and reducing the prevalence of diseases. For this, there should be close coordination and collaboration between the laboratory and country health programmes, especially between the department of epidemiology and the health laboratory. Laboratory effectiveness can be improved considerably through the application of well-evaluated strategies, appropriate selection of tests and application of epidemiological data. Such an approach should ensure optimal utilization of available resources for disease control and should also make laboratory reports more meaningful for those who use them (HCWs, physicians and epidemiologists).

Standardization of Equipment, Chemicals and Reagents

It is important to use good quality standard equipment and supplies in all laboratories of the network to ensure economy, reliability of results

and standardization of methods. The health laboratory service must stipulate the make and specifications of equipment to be used by the laboratories.

For proper evaluation of equipment, full product information, including operating instructions and a maintenance manual must be obtained from the manufacturers, and all new equipment should be field-tested. Guidance must be provided before purchase of new equipment. Selected equipment must meet medical and technical needs as well as environmental and working requirements, should be sturdy and capable of being operated and maintained reliably by the user. In addition, its capital and recurrent costs must be affordable. It should also be evaluated for safety, precision, accuracy, reagent needs, in-built quality control and speed.

The equipment should be selected after proper consultation with the laboratory and medical staff. The use of an 'equipment data specification sheet' will ensure that the requirements outlined above are met and that adequate product information (including instructions for use and maintenance and stocking of spare parts) is available. Every effort should be made to establish a dialogue with the manufacturers to devise better equipment according to local needs.

The quality of reagents and chemicals, and the performance of equipment should be maintained in all the laboratories of the network to ensure reliability of results and standardization of methods.

Equipment should be accepted from donor agencies only after thorough consideration and discussion, so that only appropriate equipment is accepted. It is necessary to enter into an agreement with the donor agency for supplying spare parts along with the equipment and training the staff in its use and maintenance.

The agreement should also ensure the repair of equipment by the donor agency for at least five years. If equipment is offered *ex gratia* by a manufacturer, the recipient must be made fully aware of the implications of running costs and other financial commitments.

Kits and instruments for rapid diagnostic tests must be evaluated by the central laboratory for sensitivity, specificity, reproducibility, negative and positive predictive values, clinical reliability, suitability under the local environment and conditions, simplicity and cost before introducing them in the laboratory network. Similarly, all chemicals, reagents, media and biologicals must be standardized for use in the laboratory system and reliable manufacturers identified. Selected manufacturers can then be given rate contracts for specified items. Once the equipment, chemicals, reagents, media, biologicals and diagnostic kits have been standardized, and specifications and makes evaluated, a national manual on these standards should be prepared by the central laboratory and distributed to the laboratories in the network. This should include instructions for handling biohazardous products.

Purchase and Supply Policy

The modalities of purchase and supply should be worked out in each country to ensure the ready availability of standard equipment and supplies at all levels of laboratory. The policy may vary in different countries, from bulk purchase and distribution to purchase by individual laboratories. Centralized purchasing requires a good purchase and stores department, efficient inventory control, a communication system and transport facilities. Such an arrangement should prove to be efficient and cost–effective for small countries. But in larger countries, purchases may have to be made at the central/state level as well as at the intermediate level. Since the central laboratory standardizes

The national plan should contain instructions for preventive maintenance, stocking of spare parts and training of technicians/engineers.

equipment and supplies, the different levels of laboratory should have no difficulty in purchasing them. However, supplies for peripheral laboratories should be provided from intermediate laboratories and, for some materials, even from the central laboratory.

There are two approaches for the supply of reagents: purchase of commercial reagents or local production of reagents from stock chemicals. The advantages of using commercial reagents include the ready availability of high quality reagents; but the disadvantages include high cost, inaccurate reconstitution, use after the expiry date and inappropriate storage.

The advantages of local production of reagents include lower cost, better use of local resources and less risk of reagents getting out of stock; but the disadvantages are several. For example, local production requires the availability of suitable physical facilities, a stock of chemicals in the country, distilled water of good quality, proper equipment (refrigerators, freezers, fume cupboards, analytical balances, pH meters, volumetric glassware), reliable power supply, adequate storage facilities, knowledge and practice of safety procedures, and proper education and training for the preparation of reagents. Organizational aspects that must be taken care of include ensuring that the quantities of reagents required and inventory control are sufficient for the demand (whether the production of reagents is centralized or decentralized) and ensuring that manpower and transport facilities for distribution are available.

Maintenance and Repair of Equipment

Maintenance and repair of equipment are the weakest aspects of laboratory services in most countries of the Region. A national plan should be developed which includes instructions for preventive maintenance, stocking of spare parts and training of technicians/engineers. Although manufacturers' engineers may be available in a country, and contracts for maintenance should be given to these manufacturers, there is a need to develop a national infrastructure for servicing and repair. A national manual on recommended techniques, reagents and equipment would facilitate the training of staff and improve the quality of work at all levels.

Self-reliance in Production of Equipment and Reagents

While it would be ideal to produce all equipment, reagents, chemicals, media and biologicals locally in a country, this may not be possible. Most instruments produced by big companies are designed to meet the requirements of industrialized countries, whose climatic and other conditions are quite different from those in developing countries.

Therefore, serious attempts should be made to become as self-sufficient as possible in the production of equipment and other supplies. The advantages include lower cost, better use of local resources and perhaps a smaller risk that supplies will be out of stock.

The constraints are that local production requires technological capability, industrial infrastructure and other prerequisites which must be fulfilled, otherwise the finished products will be substandard and give erroneous results.

Human Resource Planning, Production and Management

In laboratory services it is the quality of personnel that ultimately determines the effectiveness and efficiency of the service. The mere availability of physical facilities, equipment and supplies cannot ensure quality of service. Therefore, the central laboratory must recruit and train good quality laboratory scientists and technicians.

Human resource planning

Planning must be done carefully in order to ensure that the different categories of laboratory staff are recruited in adequate numbers to fill vacant positions and to provide for retirement/loss of staff as well as future expansion. Initially, the total requirement for laboratory personnel of all categories (medical, science graduates, technicians and para-medical) must be assessed. This will obviously depend on the number of laboratories, their staffing patterns, the future development/expansion of laboratories, and loss due to retirement.

Human resource production

Medical graduates

Specialists in laboratory medicine

Specialists in laboratory medicine will work in the various sections at the central/state laboratory level and as heads of various sections at the intermediate laboratory level. If the necessary universities and medical colleges required for training laboratory medicine specialists are lacking in a country, neighbouring countries should be able to help. These laboratory specialists should hold an MD degree in one of the branches of laboratory medicine, viz. pathology, microbiology or biochemistry.

There should be communication between the health laboratory service and universities or the medical education regulatory body of the country to ensure that proper training in public health laboratory work is given during the MD course.

Medical officers at peripheral level

Medical officers at the peripheral level should initially be given orientation courses in laboratory work and receive refresher courses every 3–4 years. This is essential for supervision of the day-to-day work of the laboratory. Although medical officers are responsible for the administrative supervision of peripheral laboratories, technical supervision of these laboratories should be the responsibility of intermediate laboratories.

Science graduates

These are graduates or PhDs in laboratory sciences, such as medical laboratory technology, microbiology and biochemistry. The graduates function as specialists and may become heads of sections in the central/state laboratory depending on experience, training and performance. Science graduates with experience, especially in medical laboratory technology, will also be of great help in subordinate positions in the laboratory network.

Technical personnel

The establishment of national training centres is an important aspect of manpower development. It is better for staff to receive basic training in the country itself rather than be trained in artificial conditions abroad. Medical colleges and universities may help the central/state level laboratories to this end. Training abroad should be considered only for highly qualified staff, and even then, only if local facilities are inadequate or do not exist.

Planning must be done carefully in order to ensure that the different categories of laboratory staff are recruited in adequate numbers to fill vacant positions and to provide for retirement/loss of staff as well as future expansion.

Regular refresher courses must be arranged for laboratory technicians to further develop the knowledge and skills required by the health service.

The training requirements include courses for the production of technical staff, refresher courses for laboratory technicians who are already in service, higher training for specialized laboratory work and training for paramedical staff (health workers) who carry out basic laboratory work at the periphery where no laboratory technicians are posted.

Laboratory technicians

Laboratory technicians should be recruited after completing high school and, if possible, their motivation should be assessed. The two years of training should be oriented according to the health priorities of the country, and include all branches of laboratory medicine.

The training should be both theoretical and practical and should enable the trainees to perform their duties with responsibility. Laboratory technicians should also understand the clinical purpose of tests performed, and appreciate the role of the laboratory in the health service. Such training should enable physicians assigned to peripheral areas to have confidence in the laboratory support they receive.

In addition to testing methodology and its purpose, training for laboratory technicians should include:

- preventive maintenance of equipment;
- quality control and biosafety;
- specimen collection and packaging;
- simple managerial skills, e.g. record-keeping, ordering;
- knowledge of sterile techniques;
- simple mathematics;
- elements of epidemiological field research; and
- health education.

Refresher courses (continuing education)

Regular refresher courses must be arranged for laboratory technicians. These are essential to further develop the knowledge and skills required by the health service. Apart from refresher courses, alternative approaches of continuing education may also be utilized, e.g. distribution of written documents, and audio and audiovisual aids.

Higher training for specialized laboratory work

There should be provision for higher-level training in specialized areas of laboratory work. Suitable technicians with adequate experience should be selected to train in special areas.

Paramedicals

Each country should develop a scheme for training paramedicals in elementary laboratory work, so that laboratory tests can be carried out at the peripheral level.

Human resource management

All the staff appointed to the services should be full-time, and provision should be made for their future promotion after acquiring the necessary experience and training. Measures should be taken to provide job satisfaction for laboratory personnel, particularly those at the periphery. Due attention should be paid to salary structure, supervision, appreciation of good work including institution of awards for excellent work, distribution of monthly or quarterly reports of interest by the central laboratory, continuing education and opportunities for promotion.

The possibilities for promotion of peripheral laboratory workers are particularly excellent, and competent individuals must be considered seriously, otherwise they may become frustrated and lose motivation. Suitable mechanisms should be worked out for this purpose.

Standard Laboratory Techniques

The development of new instruments and the availability of new chromogens, chemicals and standards have given rise to a confusing number of methods for the determination of every substance. Many estimations, particularly those of enzymes, can be expressed in different units, and laboratories work at different temperatures and with different substrates, making the situation even more difficult.

This lack of standardization has implications for physicians, who cannot interpret results when unfamiliar reference values are used and also cannot compare results from different laboratories. This creates difficulties in the quality control of laboratory services.

The central laboratory should standardize the methods used in the laboratory network enabling uniform techniques to be followed throughout the country. This should simplify training, promote economy and efficiency, and allow the use of standardized equipment and the results of different laboratories to be compared.

Laboratory Manuals

The WHO Manual of Basic Techniques for a Health Laboratory is being revised and, when available, the techniques described can be adapted by the central laboratory of each country according to its needs.

A single manual for peripheral laboratory services may suffice, but for intermediate and central/state laboratories, subject-wise manuals should be produced. The manuals should include laboratory techniques which concern the various vertical health programmes, and chapters on preventive maintenance of equipment, quality assurance, biosafety, specimen collection and packaging and simple managerial skills, e.g. record-keeping and ordering supplies.

Manuals for peripheral laboratory services should be prepared after due consultation with intermediate and peripheral laboratories, and are best prepared in the local language. An atlas or bench-aid consisting of colour photomicrographs will be valuable in diagnosing causes of anaemia from red cell morphology and for identifying malaria and other blood parasites and intestinal parasites.

Supervision should be supportive in nature and carried out by means of supervisory visits, training, continuing education, logistic support, quality control and guidance in the daily activities of lower level laboratory workers.

Supervision and Support

Supervision of lower level laboratories by a high level of laboratory is an important prerequisite for effective and efficient functioning of laboratory services. Supervision here has a broader meaning than simply controlling the activities of lower level laboratories. It should be supportive in nature and carried out by means of supervisory visits, training, continuing education, logistic support, quality control and guidance in the daily activities of lower level laboratory workers.

Higher level laboratory personnel should make at least two to three visits every year to lower level laboratories, and these visits should be coupled with checks on quality control activities, checking the inventory and condition of supplies and equipment, discussing problems, and answering technical questions. These visits/discussions should help to maintain the interest and provide motivation for laboratory workers.

Referral System

A referral system for sending samples from lower to higher level laboratories and for timely reporting and dispatch of test results from higher to lower level laboratories should be developed. Clear instructions and guidelines as well as logistic support from higher level laboratories are required with regard

to sending specimens. Laboratories should be aware of any specific national and international regulations concerning the use of postal services for transporting infectious materials.

The central/state or intermediate laboratory, as the case may be, should supply requisition forms, media, sample collecting containers and pertinent information on collection methods to lower level laboratories. The collection and dispatch of specimens should take place at all levels of the laboratory network, and it is important to adhere to biosafety practices while packing and sending potentially infectious specimens.

Additional research and information will be needed on:

(a) methods for shipping specimens which will allow them to withstand the rigours of transport and still yield reliable results; and

(b) more rapid, inexpensive and simple tests which can be carried out at the peripheral level, as referral may not always be practicable due to poor communication and transport facilities.

The referral system will only work if the results of tests are sent back quickly by the referral laboratory so that the referring laboratory can use them gainfully.

Always adhere to biosafety practices while packing and sending potentially infectious specimens.

Reports and Information

Information should flow from peripheral and intermediate laboratories to central/state level laboratories. In addition to data on diagnostic activities, reports should include information necessary for epidemiological surveillance, quality control and stock levels of various supplies and consumables. The central laboratory must develop a standardized format for reporting.

Reporting on a monthly basis should meet the needs of routine activities, while for supplies a six-monthly report of stock levels should be sufficient. Test results of specified epidemiological significance should be reported immediately. Similarly, any supplies accidentally destroyed or depleted or a sudden breakdown in equipment should immediately be brought to the notice of the central laboratory.

There should be a flow of information in the other direction as well. The central laboratory must send feedback from epidemiological and laboratory data and other information to intermediate and all peripheral laboratories in the network. This can be in the form of a quarterly/six-monthly newsletter and should contain information on how the laboratories are operating in comparison with other laboratories, new methods and reagents, disease problems, epidemiological news, interesting test results from laboratories in the network and career opportunities.

Communication and Transport Facilities

A good communication system is imperative for conveying urgent messages, instructions, unusual happenings, consultations and dialogues between laboratories of the network. Also, an efficient transport system is essential for regular logistic support, referrals, sending reports and information and for supervision. Although these facilities largely depend on the general development of transport and telecommunication facilities in the country, and on the type of terrain and geographical features, it is essential that adequate funds be provided for meeting the expenses of transport and telecommunication. Very often, adequate funds are not given for these indispensable activities.

Quality Assurance Programme

Quality assurance in laboratories of the network is essential for providing reliable, precise and accurate results of investigations in support of optimal patient care, epidemiological surveillance and research. Quality assurance improves test reliability by helping to minimize the variability arising from biological or analytical sources inherent in all types of examination. Therefore, the central laboratory must develop a quality assurance programme (QAP) for the laboratories of the network. A wide spectrum of quality improving activities is needed to ensure the reliability and usefulness of laboratory investigations. Quality assurance embraces many factors, including good laboratory practice, internal quality control and external quality assessment. These measures require training of laboratory staff, good communication between laboratory staff and other health workers and maintaining high standards in laboratory techniques.

Internal Quality Control (IQC)

Internal quality control is the set of procedures undertaken by the staff of a laboratory for continuously assessing laboratory work, including the results, and deciding whether the results are reliable enough to be released.

These procedures have an immediate effect on the laboratory's activities and should actually control, as opposed to merely examine, the laboratory's output. The main object of IQC is to ensure day-to-day consistency.

General quality assurance measures

- Selecting appropriate investigations and identifying the specimens required;
- Proficient collection of specimens under appropriate, well-defined conditions and a reliable system for proper labelling and identification of specimens;
- Transport of specimens to the laboratory in such a way that no critical changes occur; selection of appropriate and reliable test techniques; use of reliable and well-maintained equipment and reagents; availability of trained and competent staff; and
- Working in a safe, well-lighted and appropriate environment; the use of well documented test procedures; accurate recording and reporting of laboratory results and correct interpretation by appropriate persons for action.

External quality assessment

The term 'external quality assessment' (EQA) should be used in preference to 'external quality control'. EQA refers to a system of objectively assessing a laboratory's performance through an external agency. This assessment is thus retrospective, and different laboratories can be compared. EQA will be successful only if it is coupled with education and training of laboratory staff in quality assurance, effective IQC and on-site visits and supervision.

The principles of quality assurance and IQC should form an integral part of all standard operating procedures in the health laboratory network. Both IQC and EQA should be implemented at all echelons of the laboratory network. All laboratories, whether public or private, should be encouraged to participate in the QAP.

At the peripheral laboratory service level, the methodology of the QAP should be very simple and adapted to local possibilities and resources. During visits by supervisors, careful observation, checking of methods, reagents and equipment and advising and teaching can be effective in identifying and correcting faults and difficulties. The checking of randomly selected patient specimens or slides at the time of the visit, or the dispatch of a

certain number of positive and negative slides to the intermediate laboratory or in the opposite direction, is also a useful procedure.

There is an urgent need to develop and start a national QAP, at least for the disciplines of clinical chemistry, haematology and clinical microbiology, in all the laboratories of the service. Guidelines for quality assurance in laboratory medicine are given in Part III of this document.

Sequence of events under EQA

- The central laboratory or other organizing central agency sends identical specimens to participating laboratories;
- Results from participating laboratories are sent to the organizing centre;
- Statistical analysis of the results is carried out;
- Reports are sent back by the organizing centre to all participating laboratories;
- Assessment of performance of the participating laboratories is conducted; and finally
- Remedial measures are taken.

12. Laboratory Safety Programme

THE HEALTH LABORATORY can be a hazardous workplace unless due precautions are taken to avoid injuries and accidents. Measures must be taken to minimize the damage incurred through accidents.

The responsibility of the laboratory safety programme should be shared between the management and the workforce and, while definition, promotion and enforcement of safety, including establishment of a laboratory safety committee, are management responsibilities, prevention rests primarily with the workforce.

All workers in the laboratory have a responsibility to themselves and to others, and all should be given guidelines on biosafety, and encouraged to follow and develop a system in which they remind each other of careless behaviour.

The management should initially constitute a Laboratory Safety Committee and designate a Laboratory Safety Officer. The Laboratory Safety Officer should be a suitably experienced person from among the laboratory staff.

The Safety Committee should meet regularly, at least once in three months, and its chairperson should be the head of the laboratory.

Safety Policy

The responsibility for developing and enforcing the safety policy rests with the management, and the policy is framed after discussions with the safety officer, the safety committee, supervisors and employees.

The objectives are to:

- provide safe premises and working conditions;
- look after staff welfare;
- provide written documents about measures to be taken to prevent all types of hazard (fire, electrical, chemical, gaseous, radiological, microbiological, physical, noise and vibration); and

- conduct training programmes in laboratory safety procedures for all new staff.

The safety policy must be applied to and practised by everyone. Failure to observe the rules should lead to reprimand or even suspension of an employee.

Safety Programme

The Safety Programme has several important components:

Design of premises

Proper attention must be paid to the size of the premises, fittings and furnishings, work surfaces, flooring of the laboratory, lighting, ventilation, storage and security.

Safe management of the environment

The fire safety programme should include:

- provision of special storage facilities for flammable substances;
- fume cupboards;
- a fire detection and alarm system;
- fire extinguishers;
- staff training in fire containment;
- good electrical, chemical and general maintenance; and
- correct waste disposal.

The electrical safety programme should insist on manufacturer certification of equipment, proper installation, proper wiring, provision of good quality fittings and maintenance. There should be provision for automatic cut-out of dangerous voltage fluctuations, an emergency electricity supply for essential equipment and standby lighting.

Gases for fixed items of equipment should be connected by a drop level safety cock to permanent pipework using screwed union connectors. Bunsen burners must also be controlled by a safety cock. The gas supply inside microbiological safety cabinets must be controlled by solenoid valves which allow the gas to pass only when the fan is switched on.

Compressed gases present several hazards and their use must be avoided if possible; and compressed gas cylinders must be kept and secured in an external weather-proof store. Installation of piped compressed air, vacuum or gas systems must be undertaken by a specialist contractor.

Noise and vibration produced by equipment should be controlled. The noise level should not exceed 70 dBA, particularly if the noise is sustained.

Microbiological safety can be ensured by 'containment' facilities compatible with the level of microbiological hazard in the laboratory, such as gloves, protective clothing, goggles, disinfectants for decontamination and microbiology safety cabinets. When there is risk of droplet spread (e.g. through vigorous shaking) a class I microbiology safety cabinet must be used. Exhaust from the cabinet should lead directly to the outside atmosphere or into the laboratory air extraction system. A high efficiency particulate air (HEPA) filter must be incorporated.

Laboratory hazards can be grouped into four classes

- ***Biological hazards*** include infection or intoxication caused by biological material. Some microorganisms are highly infectious.
- ***Chemical hazards*** include contact with toxic, carcinogenic, corrosive and flammable substances.
- ***Radiological hazards*** due to radiation exposure have a long-term effect.
- ***Physical hazards*** include electrocution, falls, needle-stick injury, cuts from broken glass, burns from hot objects, cold injury, eye damage from ultraviolet rays and injuries from fire.

The laboratory safety programme should include

- Establishment of a safety policy.
- Development of a safety programme to implement the safety policy.
- Monitoring the implementation of the safety programme.
- Establishment of a safety management system.

Attention must be paid to proper transport, storage and disposal facilities that provide safety from chemical hazards. For radiation safety, work involving radioactive material should be carried out in designated laboratories, under the direction of fully trained laboratory staff. There should be provision of monitoring devices capable of detecting contamination of work areas.

Documentation of safety guidelines

Laboratory safety guidelines should be written in detail, either in a safety manual or as part of the procedures manual. Specific guidelines that describe the manner in which hazardous materials should be handled, precautions, rules and code of conduct to be followed in daily routine, the type of protective clothing or eye wear that must be worn and when to wear it and the procedures to be followed in case of inadvertent exposure or injury, must be put in writing and followed by all.

General guidelines should also include details of rules on personal hygiene, prohibition of eating, drinking and smoking in the laboratory, safe pipetting procedure, waste disposal, proper use of centrifuges, decontamination procedures following breakage/spillage of specimens, fire prevention measures, and electrical, microbiological, chemical and radiation safety procedures.

Staff training

If training of staff is effective, they will have good appreciation and knowledge of the risks involved in laboratory work. The laboratory safety programme must prevent injury or sickness of laboratory personnel, and must protect other persons (e.g. tradesmen, service engineers and patients). Staff should also be trained in safety procedures which prevent accidental HIV infection. Laboratory personnel have a responsibility to adhere to the programme, and must avoid complacency.

Monitoring the Implementation of the Safety Programme

Once framed, the safety programme must be implemented. It is more economical to invest in safety equipment and personnel resources than incur costs from loss of time,

Staff welfare

- **Employment health programme**

  Pre-employment medical examinations should be carried out, and all laboratory personnel should be offered vaccination against hepatitis B. The need for vaccination against diphtheria, tetanus and poliomyelitis should be determined by local circumstances. Medical facilities must be provided to all laboratory staff in case of sickness and accidents.

- **Accident reporting**

  All incidents occurring in the laboratory premises which affect the health of employees should be recorded in detail. They must be investigated by the safety officer and reviewed by the safety committee.

- **Provision of first aid**

  A well-equipped first-aid box should be available, and several staff members should be trained in first-aid procedures.

wages, health care expenses and property damage.

The safety officer along with the supervisor, must inspect all laboratories at least once in three months. A checklist should be prepared so that any deficiencies can be corrected. The safety officer should keep records of all safety measures.

All employees must be educated, trained and motivated to follow the safety procedures, use safety equipment and report incidents/accidents/injuries. Employees should also inform the safety officer about any hazards they find in their work environment.

A safety management system is required for identifying, analysing, resolving and evaluating biosafety-related problems in the workplace.

The safety committee should meet regularly at three-monthly intervals to receive feedback on safety activities.

Safety Management System

Safety management is a continuous and dynamic process. Whenever an incident occurs, it should be analysed for the reasons of its occurrence, and precautions should be determined to avoid such incidents in future. There should be an evaluation system to ascertain whether the safety measures have been implemented, i.e. there should be a system to carry out identification, analysis, resolution and evaluation of problems. Constant vigilance and review of practices in the workplace are necessary to protect staff from hazards.

13. Advisory Committee

It is useful to have an Advisory Committee. However, careful attention must be paid to the membership of this committee which should include an adequate number of technically-qualified experts in laboratory medicine and public health. The members of the committee should be drawn from universities, public health departments, hospitals, laboratories, veterinary services, national research councils, directorates of health services, and directors of quality control, taking care that adequate laboratory expertise is available to advise on technical matters.

The committee should give advice on policy matters, coordinate the work of the various organizations in the health system and purchase equipment and kits.

Sub-committees may be formed to discuss and clarify technical and scientific matters. If the need arises, there should also be a provision to co-opt members such as engineers for sub-committee meetings on purchasing and finance personnel for discussions on funding.

The safety officer along with the supervisor, must inspect all laboratories at least once in three months.

Functions of the Advisory Committee

1. **Advise on policy matters.**
2. **Coordinate the work of various organizations in the health system.**
3. **Purchase equipment and kits.**

14. Evaluation and Modification of the Health Laboratory Services

LABORATORY SERVICES provide support to all health care activities and should, therefore, be evaluated in the context of the entire health care system. Evaluation is a continuous and dynamic process. Its aim is to determine whether the health laboratory service has achieved its basic objectives, the quality of its work, and whether the service is relevant for the health needs of the country, is cost–effective and fully satisfies the user. Evaluation allows suitable modifications to be carried out in the service to make it more satisfactory. Evaluation mainly concerns two aspects—management and technical—and although accurate evaluation of laboratory services is extremely difficult, the most effective approach is to prepare an 'activity report' on a six-monthly or annual basis.

Other factors to be considered include: the composition, responsibilities and frequency of meetings of the Advisory Committee; linkages with other national programmes such as primary health care, tuberculosis, leprosy and malaria control programmes: surveillance of communicable and noncommunicable diseases; other related laboratories, e.g. veterinary, sanitary engineering, industrial and environmental, with international centres and reference laboratories. A simple way of evaluation is to collect all this information periodically (annually) on a form. However, it is better to supplement these details by periodic supervisory visits to gather first-hand information.

Management

The factors to be considered to evaluate whether the service is properly managed include:

- realistic planning and balanced staffing;
- ensuring that the architecture is adapted to the geographic and climatic conditions;
- selection, assessment, maintenance and repair of equipment;
- logistic support and supplies given by higher level laboratories to lower level laboratories;
- ensuring a well-organized laboratory safety system;
- maintenance of records and the filing system;
- request report forms;
- monthly/yearly statistical reports;
- guidelines for laboratory procedures;
- reasonable storage facilities; and
- making transport and communication facilities available for referrals and reports.

Technical

The factors to be taken into account include:

- the complete workload;
- referrals sent/received;
- the time taken to prepare and send the reports of referrals;
- laboratory procedures (whether manual, mechanized or automated);
- the availability of laboratory manuals at all echelons of laboratory service;
- the QA programme in the network;
- the hierarchy and responsibilities of all categories of staff;
- training facilities, reports, laboratory errors, unusual problems encountered;
- addition of new equipment or technology;
- special problems investigated; and
- credibility, clinical relevance of tests offered and utilization of results by consumers.

15. Impact of Implementation of Guidelines on Health Laboratory Services

A HEALTH LABORATORY Service, as outlined above, will be able to provide integrated health care in the context of PHC. The range of activities, from the sophisticated modern facilities available in central and regional laboratories to the 'simple' approach adopted at the periphery, helps to make the service an asset to the health system of a developing country. Coordination and networking through supportive supervision at the various levels will bridge the gap that exists between the clinical and public health approaches to health care in developing countries, and presents a forum for total integration of clinical and public health laboratory services into PHC. For the successful implementation of PHC, a self-financing approach is being introduced in many countries. Self-financing leads to community participation with total involvement and commitment, and also gives sustainability to laboratories supporting PHC.

Self-financing leads to community participation with total involvement and commitment, and also gives sustainability to laboratories supporting PHC.

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Annex 1

Suggested List of Laboratory Tests for Different Levels of Laboratory

Level 1	**Peripheral laboratory**
Level 2	**Intermediate laboratory**
Level 3	**Central/State/Provincial laboratory**

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Microbiological				
Bacteriological				
Unstained wet preparations	Pus cells, RBCs	+	+	+
Urine deposit	*T. pallidum*	–	–	–
Darkfield illumination				
Ulcer exudate				
Stained smears (Gram, Albert, Ziehl-Neelsen)				
Nasopharynx and throat	Diphtheria, Vincent's angina	+	+	+
Sputum	Tuberculosis, pneumonia	+	+	+
CSF	Meningitis (pyogenic and tuberculous)	+	+	+
Skin and nasal	Leprosy	+	+	+
Gastric washing	Tuberculosis	–	+	+
Urethra/vagina	Gonorrhoea	+	+	+
Wounds	Clostridia/other organisms	+	+	+

C	*Collection of specimens and referral to next highest level laboratory*
P	*Preliminary culture and identification*
cf	*Confirmation, full identification, typing and standardized sensitivity testing*
+	*Test done*
(+)	*Optional, if feasible*
–	*Not done*

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Cultures				
Nasopharynx/throat	*Corynebacterium diphtheriae, Streptococcus pyogenes*	–	P	cf
Sputum	AFB, cocci, others	–	P	cf
CSF	AFB, cocci, *Haemophilus influenzae*	–	P	cf
Exudate/pus	Bacterial infections	–	P	cf
Blood	*Salmonella typhi*, other salmonellae, *Brucella*	–	P	cf
	Streptococci, pneumococci meningococci, *Haemophilus influenzae, Leptospira tularensis*	–	C	P, cf
Gastric washing	AFB	–	P	cf
Urethral/vaginal	*Neisseria gonorrhoeae*	–	P	cf
	Chlamydia	–	C	P, cf
	Haemophilus ducreyi	–	P	cf
Faeces	*Salmonella*	–	P	cf
	Shigella	–	P	cf
	Vibrio cholerae	–	P	cf
	Escherichia coli and others	–	P	cf
	Food poisoning bacteria	–	P	cf
Urine	Pyogenic organisms	–	P	cf
	AFB	–	P	cf
Anaerobic cultures	*Clostridia* and others	–	P	cf
Antibiotic sensitivity tests				
Mycobacterium		–	–	+
Other bacteria		–	+	+
Blood levels of aminoglycosides		–	+	+
Pathogenicity tests				
Laboratory animal inoculations	Various organisms	–	(+)	+
Phage typing/ Complete serotyping	*Salmonella, Staphylococcus aureus*, streptococci, *Escherichia coli*	–	–	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Serological tests	Enteric fever (Widal)	C	+	+
	Brucella (tube agglutination)	C	+	+
	Rickettsia (Weil–Felix)	C	+	+
	C-reactive protein	–	+	+
	ASLO	C	+	+
	Anti DNAseB	C	C	+
	Paul Bunnel	–	+	+
	Rheumatoid factor	–	+	+
	Cold agglutinins	–	+	+
	VDRL	–	+	+
	TPHA	–	(+)	+
	FTA-Abs	–	C	+
	Leptospira	–	C	+
	Immunoglobulin levels	–	(+)	+
Virological				
Rapid diagnostic tests (Latex, Dipstick ELISA, etc.)	Direct antigen detection (HBsAg, RSV, Rotavirus, Influenza A, Herpes, CMV, etc.)	(+)	+	+
Fluorescent antibody staining				
Post-mortem diagnosis throat, nasopharyngeal	Rabies in dogs	–	C	+
	Respiratory viruses	–	–	+
Blood (ELISA)	Detection of HBsAg and HIV antibodies	C	+	+, cf
Serological surveys	JE, dengue, haemorrhagic fever, polio, measles, HBV, Rubella	C	C	+
Egg inoculation/tissue culture/animal inoculation	Isolation of viral agents	C	C	+
Neutralization, haemagglutination Inhibition for identification	Viral agents	C	C	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Mycological				
Wood's light examination	Dermatophytes	(+)	+	+
Wet mounts				
Hair	Piedra	(+)	+	+
Hair, skin, nails (KOH)	Dermatophytes	+	+	+
Skin	Chromoblastomycosis	–	+	+
Sputum	Rhinosporidiosis	–	+	+
Bronchial washings	Yeasts, mycelium, granules	–	+	+
Pus	Yeasts, mycelium, granules	–	+	+
Spinal fluid (India ink)	Yeasts, mycelium, granules	–	+	+
Stained smears				
Sputum (Gram and acid-fast)	Yeasts, *Actinomyces*	+	+	+
Mucous membrane scrapings (Gram, Wright)	Yeasts, *Histoplasma capsulatum*	+	+	+
Blood (Wright or Giemsa)	*Histoplasma capsulatum*	–	+	+
Bronchial washings	Yeasts, *Actinomyces*	–	+	+
Cultures, inoculation of media and identification	All pathogenic fungi	C	P	cf
Pathogenicity tests	*Cryptococcus neoformans, Actinomyces*	–	–	+
Skin tests	Histoplasmosis	–	(+)	+
Parasitological				
Wet preparation				
Faeces	Exudate, trophozoites, ova, cysts	+	+	+
Urine	Schistosomes	+	+	+
Vaginal swab	*Trichomonas*	+	+	+
Sputum	*Paragonimus*	C	+	+
Blood	Microfilariae	(+)	+	+
Rectal biopsy or aspirates	*Entamoeba histolytica*, Schistosomes	–	+	+
Stained thin and thick blood film	Malaria, filariasis, leishmaniasis	+	+	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Stained faecal smears and rectal aspirates	*Entamoeba histolytica*, Schistosomes	–	+	+
Concentration				
Faeces	Ova and cysts	–	+	+
Blood	Microfilariae	–	+	+
Egg counts in faeces	Estimation of worm loads	–	+	+
Examination of arthropods, snails, etc.	Identification of vectors intermediate hosts etc.	–	–	–
Serological tests	Various parasitic diseases	–	–	+
Complement fixation, latex agglutination, Bentonite flocculation, ELISA, dye test for Toxoplasma, Haemagglutination, etc.				
Casoni's test	Hydatid disease	–	+	+
Haematology				
Haemoglobin (Hb) concentration (oxyhaemoglobin at Level 1, haemiglobin cyanide at Levels 2 and 3)		+	+	+
White cell count		+	+	+
Red blood cell count (using automated or semi-automated equipment)		–	(+)	+
Erythrocyte sedimentation		+	+	+
Haematocrit		+	+	+
Thin blood films (differential WBC; RBC morphology)		+	+	+
Platelet count		+	+	+
Reticulocyte count		+	+	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Eosinophil count		+	+	+
Bleeding time		+	+	+
Clotting time		+	+	+
Bone marrow examination		–	+	+
Prothrombin time		–	+	+
Sickle cell screening test		–	+	+
Partial thromboplastin time		–	+	+
Thrombin clotting time		–	+	+
Fibrinogen estimation		–	(+)	+
Fibrin degradation products		–	(+)	+
Coagulation factor assays		–	–	+
Blood grouping [(ABO; Rh(D)]		–	+	+
Crossmatching		–	+	+
Direct antiglobulin test		–	+	+
Antibody screen		–	+	+
Antibody identification		–	(+)	+
Serum or plasma iron (total iron binding capacity)		–	(+)	+
Serum ferritin		–	(+)	+
Serum vitamin B_{12}		–	–	+
Plasma and red cell folate		–	+	+
special stains—red cells		–	+	+
—white cells		–	+	+
Serum haptoglobin		–	–	+
Osmotic fragility		–	+	+
LE cell phenomenon		–	+	+
Hb electrophoresis		–	(+)	+
Hb F estimation		–	(+)	+
HbA_2 estimation		–	(+)	+
G6PD screening test		–	+	+
Red cell enzyme assays (e.g. G6PD)		–	–	+
Detection of Hb derivatives		–	–	+
Estimation of Hb derivatives		–	–	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Clinical Chemistry				
Urine				
Physical examination, specific gravity, etc.		+	+	+
Microscopy of deposit		+	+	+
Protein		+	+	+
Glucose		+	+	+
Pentoses		–	+	+
Ketones		+	+	+
Bile pigments		+	+	+
Urobilin		+	+	+
Urobilinogen		+	+	+
17-Ketosteroids		–	–	+
Urinary calculi		–	(+)	+
Pregnancy test		(+)	+	+
Sperm examination		(+)	+	+
Nitrite		–	+	+
Drugs of abuse		–	–	+
Blood/Serum/Plasma				
Calcium		–	(+)	+
Chloride		–	(+)	+
Cholesterol		–	+	+
Creatinine		–	–	+
Creatine		–	(+)	+
Glucose		–	+	+
Phosphorus		–	(+)	+
Total proteins		–	+	+
Blood urea nitrogen		–	+	+
Uric acid		–	+	+
Globulin		–	+	+
Albumin		–	+	+
Alkaline phosphatase		–	+	+
Acid phosphate		–	+	+
Carbonate/total CO_2		–	+	+
Glucose and insulin tolerance, etc.		–	(+)	+
pH, blood and plasma		–	(+)	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Non-protein nitrogen		–	(+)	+
Bromides		–	–	+
Magnesium		–	–	+
Potassium		–	–	+
Sodium		–	–	+
Protein-bound iodine		–	(+)	+
Copper		–	–	+
Thiocyanates		–	–	+
Transaminase (glutamic oxaloacetic and glutamic pyruvic)		–	+	+
Other enzymatic tests		–	–	+
Phenolsulfonphthalein test		–	(+)	+
Icteric index		–	+	+
Bilirubin		–	+	+
Thymol turbidity test		–	+	+
Bromsulphalein test		–	(+)	+
Phenol turbidity test		–	(+)	+
Zinc turbidity test		–	(+)	+
Cephalin–cholesterol flocculation test		–	(+)	+
Toxicological markers		–	–	+
Therapeutic drug monitoring		–	–	+
CSF				
Glucose		–	+	+
Protein		–	+	+
Chloride		–	+	+
Globulin		–	(+)	+
Gastric and duodenal fluids				
Occult blood		–	+	+
Total acidity and free HCl		–	+	+
Pancreatic enzymes		–	–	+
Faeces				
Occult blood		–	+	+

Procedure/Specimen	Disease/Organism	Level 1	Level 2	Level 3
Histopathological and Cytological Examinations				
Autopsy		–	+	+
Biopsy, organs, tissues		–	+	+ /cf
Pap smears, FNAC, etc.		–	+	+
Forensic Examinations				
Autopsy		–	+	+ /cf
Immuno-serological tests		–	C	+
Special tests		–	C	+
Clinical toxicological tests, gases and poisons		C	C	+
Hygiene Laboratory Services				
Bacteriological and chemical tests for water, milk, dairy products, food		–	+	+

Part II

Appropriate Technology for Health Care in the South-East Asia Region

1. Introduction

WHO PRIORITIES in the 1990s are to improve and promote essential health technology applicable at PHC level and the immediate supporting level of the health system to accelerate the process for HFA 2000. The World Development Report 1993 (Investing in Health) refers to provision of 'essential clinical services and public health packages' in the countries, and essential health technology may be regarded as one of the main components of these packages.

The major thrust of WHO is principally based on the concept of essential health technology (EHT) and on making it accessible to the people. The essential component of EHT is promotion of health through prevention and control of prevalent diseases, for which diagnosis is an important activity and should be provided at the basic health care level. Diagnostic technology should be available in an appropriate manner for basic health care. It is essential to assess EHT and develop appropriate technology in countries through technology transfer.

Appropriate technology is the adaptation to local circumstances and conditions of knowledge and skills which are scientifically sound and acceptable to those who apply them and to those for whom they are used.

The disease burden in developing countries is much higher than that in developed economies because of the prevalence of communicable diseases.

The World Development Report 1993 focused sharply on the fact that the disease burden in developing countries is much higher than that in developed economies because of the prevalence of communicable diseases. An overwhelming number of deaths (40%) in developing countries are due to communicable diseases.

Appropriate technology is the adaptation to local circumstances and conditions of knowledge and skills which are scientifically sound and acceptable to those who apply them and to those for whom they are used. Appropriate technology for laboratory tests calls for tests which are cost-effective, easily and rapidly performed, and adaptable, especially in peripheral laboratories in support of PHC.

Delay in diagnosis of acute infection is disastrous, both for the patient and the people who are exposed to the infection. Conventional microbiological diagnostic techniques, though specific and sensitive, are often tedious, difficult, time-consuming and impossible to perform in some laboratories. These

methods are inherently slow as they are based on the rate of growth of bacteria.

The last two decades have witnessed rapid advances that have led to the availability of innumerable rapid diagnostic laboratory procedures for demonstrating the presence of bacterial antigens or antibodies directly in the pathological material without its culture. The progress in diagnosis of viral infections through simplified procedures for antibody and antigen detection has been remarkable.

Until the 1970s, rapid virus diagnosis was not practical as facilities for tissue culture and other laboratory procedures were costly, and needed special equipment and well-designed facilities. Even today, comprehensive facilities for diagnosis of viral infections exist in very few laboratories in developing countries, and the benefits of diagnosis are not available to the majority of the population.

Recently developed tests for rapid diagnosis of bacterial, viral, parasitic and mycotic infections include coagglutination, latex fixation, enzyme-linked immunosorbent assay (ELISA), immunochromatographic assay (ICA), fluorescent antibody test (FAT) and counter immuno-electrophoresis (CIE). With the advent of monoclonal antibodies, the sensitivity of enzyme immunoassay (EIA) increased a thousand-fold and antigens can now be detected in picogram quantities. Similarly, the use of purified recombinant antigens in enzyme immunoassay systems allows detection of antibodies in nanogram or picogram quantities.

In developing countries, the benefits of laboratory diagnosis are not available to the majority of the population.

Agglutination tests, which use coated latex or gelatin particles, are less sensitive than enzyme immunoassays, but are simpler to perform than the conventional ELISA techniques. Dot blot ELISA test is also simple to perform and has high sensitivity. Some of these, such as the dipstick ELISA, ICA, coagglutination and latex fixation tests, are feasible at the PHC level as they do not require elaborate equipment, methodology and technical expertise.

Most of these techniques are sufficiently sensitive and specific and the pathogen can be identified within minutes without actually isolating the organism—a time-consuming and tedious process.

The shelf-life of currently available test kits is usually one year and they must be stored in a refrigerator. Research is being conducted to produce thermostable test kits with longer shelf-lives that can withstand field conditions. WHO has developed a simple haemoglobin colour scale to detect anaemia with reliable estimates of its severity (i.e. masked, mild, moderate, severe) and for screening of blood donors.

Depending on the available resources and the epidemiological situation, each country should choose its own strategy regarding the introduction of rapid diagnostic technology at various levels of laboratories. WHO is endeavouring to transfer the technology for rapid diagnostic kits to Member Countries and will continue this effort in making the technology more cost–effective. With international encouragement, including technology transfer, some of the countries could become self-sufficient and produce the kits locally. Increased production would lower the cost of these kits.

Expertise to produce some of the kits for rapid diagnostics is available in certain countries. Regional collaboration would ensure the availability of cheaper but high quality kits in countries of the South-East Asia Region.

2. Conventional versus Rapid Diagnostic Technology

RAPID DIAGNOSTIC techniques cannot always replace conventional culture methods, which must be conducted for complete identification of bacterial pathogens and their antimicrobial sensitivities. These epidemiological characters of microbial pathogens are essential for disease control and can be defined only by conventional tests. The antibiotic sensitivities of bacterial pathogens cannot be ascertained by the presently available rapid diagnostic tests which are usually qualitative.

These rapid tests, however, are ideal for preliminary screening of diseases, following which a specimen should be referred to higher level laboratories for detailed investigation and antibiotic sensitivity testing.

In countries of the Region, facilities for bacterial culture may be available only in advanced level laboratories and possibly in a few intermediate level laboratories. Bacterial cultivation, though sensitive and specific, is often tedious, difficult and time-consuming, The situation is even more difficult as regards cultivation of viruses, which requires sophisticated laboratory equipment and tissue culture facilities that may be available only in a few advanced level (tertiary) institutions.

Significant advances in the development of effective antiviral agents, such as acyclovir for herpes simplex and varicella–zoster, amantadine for influenza and respiratory syncytial virus, zidovudine, zalcitabine, saquinavir, indinavir for HIV, ganciclovir for CMV and interferons for immunomodulatory therapy, have emphasized the need for rapid and reliable diagnosis of viral infections.

In most countries of the Region, communicable diseases are the major causes of morbidity and mortality. Diarrhoeal diseases, acute respiratory infections, meningitis, mycobacterial infections, sexually transmitted diseases, parasitic infections and viral infections are of great importance. Thus, the major emphasis in laboratory investigations is on microbiological infections—mostly bacterial, viral and parasitic infections.

Depending on the available resources and the epidemiological situation, each country should choose its own strategy regarding the introduction of rapid diagnostic technology at various levels of laboratories.

The conventional strategy for diagnosis consists of microscopy and cultivation of pathogens, while serological methods are resorted to for antibody detection.

Microscopy is the primary examination carried out for the demonstration of bacteria, viruses and parasites. There must be a substantial number of organisms in the specimen for a positive result (Table 1)[1].

In most countries of the Region, the use of immunofluorescence and electron microscopy is restricted and ordinary microscopy is used in most laboratories.

For bacterial infections, Gram stain is the most effective, rapid and accurate method for the diagnosis of infection in specimens from sites which are normally sterile, e.g. blood, urine (BMQ), cerebrospinal fluid (CSF), and exudates. However, from sites with normal flora, e.g. throat,

faeces, etc. microscopy may not be of much diagnostic importance. Simple immunological tests have been recently devised for direct detection of antigens in patients' specimens. In situations where direct microscopy fails to give clear results, these rapid antigen detection tests can be usefully employed.

This is a great advantage as such tests can be used even when patients have received antimicrobial treatment which would make isolation of the pathogen almost impossible. Specific laboratory diagnosis can be made, often within hours of receiving the specimen by demonstrating the specific antigen in cerebrospinal fluid, serum, exudate or urine. In cases of meningitis, pneumonia and viral infections, positive results indicate what treatment is appropriate.

Simple immunological tests have been recently devised for direct detection of antigens in patients' specimens.

Indirect evidence of infection is sought through antibody responses. Traditional tests for antibody detection include agglutination, passive agglutination, precipitation, complement fixation, and antigen neutralization (for viral infections). However, advances in antigen

Table 1
Techniques for Microscopic Examinations

Techniques	Microorganisms	Limit of detection	Time required for diagnosis
Gram stain	Bacteria	10^5–10^6 cfu/mL*	10 min
Acridine orange stain	Bacteria	10^5	10 min
Ziehl–Neelsen stain	*Mycobacteria*	10^5	60 min
Auramine stain	*Mycobacteria*	10^5–10^6	60 min
Several staining techniques	Parasites	10^5–10^6 cell/mL*	10–60 min
Auramine, modified Ziehl, or Kinyoun	*Cryptosporidium*	10^4–10^5 cell/mL*	60 min
Immunofluorescence	Miscellaneous bacteria, viruses	10^5–10^6 cfu/mL*	60–180 min
Electron microscopy	Viruses	10^4–10^5 pfu/mL*	30 min
India ink	*Cryptococcus*	10^4–10^5	30 min
Potassium hydroxide (KOH) preparation	Dermatophytes	10^2–10^3	30 min

**cfu/mL, pfu/mL, cell/mL: Number of organisms per mL of specimen.*

extraction and production of recombinant antigens have greatly increased the sensitivity of these tests.

The real breakthrough, however, came with the discovery of ELISA, which further increased the sensitivity of antigen–antibody detection. Dipstick technology has brought ELISA within the reach of peripheral laboratories.

Table 2 gives the limits of detection of antigens using different immunological techniques[1].

Table 2
Limits of detection of antigens

Immunological technique	Limit of detection	Time required for diagnosis
Coagglutination (CoA)	ng/mL	2–10 min
Latex agglutination (LA)	ng/mL	2–10 min
ELISA		
– conventional	ng/mL	2–8 hours
– dipstick		2–10 min
Counter-immunoelectrophoresis (CIE)	ng/mL	1–24 hours
Radioimmunoassay (RIA)	ng/mL	4–24 hours
Immunochromatographic assay (ICA)	ng/mL	5–10 min

3 Principles of Rapid/Simple Diagnostic Procedures

Coagglutination Technique

THE COWAN strains of *Staphylococcus aureus* have protein A on their outer surfaces. The protein A binds selectively to the Fc region of the IgG molecule and leaves the Fab region free to combine with the homologous antigen. Monoclonal antibodies or conventionally raised antisera (IgG) are used to coat *Staphylococcus aureus* and the suspensions usually retain reactivity for about one month if kept at 4 °C. The amount of antigen which can be detected by the coagglutination test within two to ten minutes is in nanogram/mL. In the test, antigen and suspension are mixed together on a slide and rapid coagglutination occurs.

The method is used for antigen detection in several bacterial infections, for example, salmonellae, shigellae, and gonococci and for grouping streptococci and typing pneumococci. Coagglutination is used directly with CSF, serum and urine for detecting the presence of bacterial antigens.

The technique can also be applied to bacteria grown on agar plates or in liquid media in pure cultures, or to their antigens. It has been found that some patients' sera react with unsensitized staphylococci and may therefore give false-positive results. This activitiy can often be removed by absorption.

Latex Agglutination

The latex agglutination test has been standardized for identification of microbial antigens as well as antibody detection. Latex particles are used as carriers of specific immunoglobulins for detection of antigens in clinical specimens. On the other hand, for detection of antibodies, latex particles serve to carry

antigens. The polystyrene latex spherical particles, of diameter 0.8–1 μ, can adsorb different types of antigens and immunoglobulins.

In order to prevent non-specific reactions when detecting antigen by the coagglutination and latex particle methods, it is essential to inactivate any IgG that may be present in the specimens. This is usually done by exposing the specimens to heat, e.g. CSF is exposed to a temperature of about 100 °C for one to four minutes.

Gelatin Particle Agglutination

Gelatin particles as carriers of specific immunoglobulins for the detection of antigens, and as carriers of antigens for the detection of antibody, have been developed by the Japanese. Gelatin particles do not manifest non-specific agglutination. The gelatin particle passive agglutination test has recently been introduced to detect antibodies against HIV and *Mycoplasma pneumoniae*, adult T cell leukaemia virus, antistreptolysin-O and antistreptokinase.

Enzyme-linked Immunosorbent Assay (ELISA)

This test relies on adsorbing material which is specific for either antigen or antibody. For instance, when antibody is adsorbed on a solid phase it will 'capture' the antigen. Antigen detection is accomplished using an enzyme-labelled antispecies globulin. The unreacted enzyme-labelled antibody is removed by washing. An appropriate substrate is added to the reaction mixture to commence the enzymatic activity, and the enzyme–substrate reaction produces a colour which is visible to the naked eye. Quantification of the colour developed is carried out spectrophotometrically in a specially designed ELISA reader. This reaction has been used extensively to detect antigen and antibody. For the detection of antigen, antibody is used for coating the wells of a microtitre plate, while for antibody detection, antigen is used for coating the wells. The different techniques are given below.

Different ELISA Techniques

- Antigen capture ELISA
- Antibody capture ELISA
- Competitive ELISA for antibody detection
- Dipstick ELISA

Antigen capture ELISA

This technique is used to detect antigen in clinical material. The wells of a microtitre plate are coated with specific antibody and the test samples are added. Unbound antigen, if any, is washed off. Enzyme-labelled antibody specific to the antigen under examination is added, following which the unbound material is washed off. The enzyme substrate is then added, and after incubation, the reaction is stopped. Colour development indicates the presence of antigen, while no colour indicates the absence of antigen.

Antibody capture ELISA

This technique is used to detect antibody in serum samples. Antigen is used to coat the wells after which the serum samples are added. Unbound material is washed off. Enzyme-labelled antibody is added and the unbound antibody later washed off. Enzyme substrate is then added and after incubation the reaction is stopped. Colour development indicates that antibody is present, while no colour indicates that antibody is absent.

Competitive ELISA for antibody detection

In this test, antigen is used for coating the wells of microtitre plates. The samples under examination and enzyme-labelled specific antibody are added simultaneously. After washing off the unbound material, the enzyme substrate is added. After

Dipstick ELISA is a rapid, simple to perform test. It is as sensitive and specific as the conventional form. In the last decade, diagnostic kits for a large number of conditions have been modified to the dipstick technology.

stopping the reaction, the colour is read. No colour indicates that antibody is present, while colour development indicates that antibody is absent.

In this test, the test antibody and specific antibody compete with each other and undiluted test serum is used.

Dipstick ELISA

This ELISA technique has been simplified through technological modification and is carried out on nitrocellulose strips. The reactions are induced by serial dipping of the nitrocellulose strips in the various reagents supplied with the kits. The test is rapid, simple to perform and is as sensitive and specific as the conventional form. It is well suited for rapid testing in field areas. In the last decade, diagnostic kits for a large number of conditions have been modified to the dipstick technology.

Liposome Immunoassay

The latest technological development involves the use of liposomes (artificial phospholipids) conjugated with antibodies or antigens for recognition of an antigen or antibody, respectively. The conjugated liposomes also contain the dye rhodamine sulphate. The antigen–antibody reaction generates a signal to release rhodamine sulphate, and thus, as in antibody–enzyme conjugate detection for production of a colour signal, the necessity of adding a substrate is eliminated.

Liposome assay technology has been useful in the diagnosis of Group A streptococci in the throat and of human chorionic gonadotrophin in the urine. The technology promises to provide the fastest test procedures and is currently being evaluated.

Immunochromatographic Assay (ICA)

Recently, sandwich immunoassay has emerged as a rapid, simple test procedure for the detection of antigen or antibody in clinical specimens. ICA utilizes a monoclonal antibody preparation conjugated with a colloidal gold dye or selenium. The antigen–antibody complex thus formed is captured by a polyclonal antibody at a different site both in reaction cards or in microtitre plates and nitrocellulose strips.

Reaction cards

The specimen under test flows through the absorbent device in the reaction card which has been impregnated with the conjugated monoclonal antibody. The sandwich of the antigen–antibody complex flows through the device and is bound by the polyclonal antibody complex, with the production of a colour.

The unbound conjugate reacts with 'control' reagents located away from the site of polyclonal antibody, to produce a colour reaction. The development of colour in 'control' indicates that the reagents used were fully functional.

Microtitre plates and nitrocellulose strips[2]

As the first step towards the formation of an antigen–antibody complex, samples under test are incubated with a monoclonal antibody conjugated to a selenium

Sandwich immunoassay has emerged as a rapid, simple test procedure for the detection of antigen or antibody in clinical specimens.

colloid in a microtitre plate. Following initial incubation, a comb-shaped device of nitrocellulose strip is dug into the wells of the microtitre plate. The sandwich migrates through the strip and is captured at the site of the polyclonal antibody. The accumulation of colloid at the capture site results in the formation of a red line, indicating a positive reaction.

Genetic probes are valuable for:

- identifying intracellular organisms in fixed cells of tissues—a technique known as *in situ* hybridization.
- identifying organisms that are difficult to grow by conventional methods, e.g. *M. leprae*.
- rapid identification of a bacterial colony or bacteriophage plaque.
- identifying extracted or amplified DNA, popularly known as dot blot hybridization.

Molecular Biology in Rapid Diagnostic Technology

There has been remarkable progress towards standardization of techniques that detect genetic material associated with infective organisms, such as DNA in bacteria, fungi and parasites and RNA in certain pathogenic viruses. Following extensive laboratory studies, it is now possible to identify very small quantities of genetic material attributable to pathogenic agents.

Polymerase chain reaction

This outstanding technique involving amplification of genetic material was first described during the 1980s. It has great potential for enabling rapid, specific and sensitive diagnosis of various infections.

Genetic probes

Another promising technique depends on the use of appropriately labelled microbial genetic material to detect pathogens in clinical specimens. Genetic probes are defined as single-stranded oligonucleotide sequences that react with complementary sequences present in the clinical material during hybridization. The probes were initially labelled with radioisotopes, although now non-isotopic probes are available.

Apart from biotin, probes have been conjugated with alkaline phosphatase and acridinium-ester. The availability of non-isotopic probes has been particularly appreciated since the end-point of the reaction is a colorimetric one.

It is expected that simplified procedures for using genetic probes will eliminate the battery of serological or physiological tests currently used and that they will be of immense utility in field laboratories.

Clinical Chemistry

Simplified procedures for biochemical investigations on clinical specimens are not new since litmus paper has been used for testing the pH of urine samples for decades. Even though synthetic dyestuffs have recently superseded the use of litmus for pH determinations, the basic technology has remained the same. The change in colour at the end-point of a reaction is the basis for instant chemical examination of urine samples.

The polymerase chain reaction has great potential for enabling rapid, specific and sensitive diagnosis of various infections.

The colour changes are monitored both qualitatively and quantitatively. It is possible to test urine specimens for pH, glucose, protein,

bilirubin and ketones simultaneously by using nitrocellulose strips. These strips have specific chemical reagents impregnated on them, and the reaction is carried out by dipping them in urine.

The changes produced in the colour of the ingredients of the nitrocellulose strips are examined by comparing the colours produced at the end of the reaction with the master chart. With the introduction of dipstick technology for blood glucose determination, the results are also read visually or are read far more accurately by means of a glucometer. Furthermore, semi-quantitative, single-step ICA tests have been standardized for assaying myoglobin or creatine kinase, and MB isoenzyme levels.

A compact reflectance photometer has been developed for estimation of various clinical chemistry values in blood. This sturdy instrument can be used at the peripheral level provided enough resources are available for purchase as well as for the constant supply of reagents.

Haematology

Diagnostic tests have been developed for various qualitative and semi-quantitative tests to provide simple procedures which can be performed with minimal technical training. The main limitation of the commercial kits is their cost, but equivalent kits could be manufactured locally.

4. Rapid Diagnostic Tests for Bacterial Infections

RAPID DIAGNOSTIC TESTS for the detection of bacterial infections were developed in the last decade. These methods allow immediate diagnosis, hence treatment can commence early.

The public health importance of these tests is inherent in the fact that communicable diseases will be recognized earlier and epidemiologists and public health authorities will be able to initiate control and containment measures earlier.

Rapid diagnostic kits are very helpful in providing early diagnosis because of their high level of sensitivity and specificity. However, one has to keep in mind the batch-to-batch variation in the kits and their shelf-life. Thus IQC must be done regularly.

New kits should first be evaluated by level III laboratories and then recommended for level I and II laboratories. One should not totally rely on the manufacturers' claims.

In the interpretation of results of tests for bacterial infections, qualitative estimation, as is available for most bacterial infections, is enough for diagnosis when the organism is always pathogenic. However, in some conditions, a carrier state also exists in which a person is infected but has no symptoms. In such cases, semi-quantitative methods should be introduced for diagnosis to gain a proper clinical perspective before the organism is labelled as pathogenic.

Rapid diagnostic kits are very helpful in providing early diagnosis because of their high level of sensitivity and specificity. However, the main limitation of commercial kits is their cost.

Bacteria Associated with Acute Upper Respiratory Tract Infections

Group A streptococci

Group A streptococci cause throat infection which, in some cases, may result in non-suppurative sequelae such as rheumatic fever and acute glomerulonephritis. Since these are serious complications, early identification of group A streptococci in throat swabs is immensely important for the control of sequelae, e.g. rheumatic fever and acute glomerulonephritis.

Research has focused on the development of a sensitive non-culture technique for the identification of group A streptococci, so that a patient with a positive throat swab can be treated immediately.

In developing countries, many children whose throat swabs are cultured conventionally do not return to collect their report and for subsequent treatment. Repeated group A streptococcal infections of the throat in rheumatic individuals further worsen the heart condition, and continual secondary penicillin prophylaxis is therefore carried out in such cases.

Non-culture techniques for the identification of group A streptococci directly from throat swabs are now available. The throat swab is processed for expressing the C carbohydrate of group A streptococci and the supernate is then tested by coagglutination[3], latex agglutination[4], ELISA, ICA and optical immunoassay.

The tests have now been developed to such a sensitivity that even 8–10 chains of streptococci present in the swab give a positive result. This test will also be important in primary prophylaxis of rheumatic fever, though it is essential for secondary prophylaxis programmes.

For diagnosis and monitoring of rheumatic heart disease, antistreptolysin (ASO) and antistreptokinase (ASK) can be detected using latex or gelatin particle agglutination. This is important in the management of rheumatic heart disease. The latex agglutination test has a sensitivity of 95%[5].

Early identification of group A streptococci in throat swabs is immensely important for the control of sequelae, e.g. rheumatic fever and acute glomerulonephritis.

Bacteria Associated with Acute Lower Respiratory Tract Infections

Acute lower respiratory tract infections are responsible for considerable deaths in countries of the Region. Most of these infections are caused by viruses and follow a self-limiting course, while studies conducted in several tropical areas have shown that life-threatening episodes are mainly caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* b. Kits for the detection of antigens of these pathogens directly in clinical samples are now available.

Bacteria Associated with Acute Meningitis

Streptococcus pneumoniae and *Haemophilus influenzae* b are also implicated in acute meningitis along with *Neisseria meningitidis*. Rapid diagnostic tests utilizing latex agglutination for the detection of antigens of these pathogens are of great value[6,7]. In most cases of acute meningitis, a direct smear of CSF is sufficient for diagnosis, unless the patient has received antibiotic treatment. In this event, the antigens may still be present in soluble form and can be detected by latex agglutination, which is more sensitive than the coagglutination test. The antigens of these organisms can also be demonstrated in urine as well as in respiratory secretions.

Group B streptococci are responsible for severe infections such as meningitis and neonatal septicaemia, and rapid detection is essential for early treatment. Excellent results have been obtained using the latex agglutination test on samples of CSF, serum, urine and respiratory secretions from cases of meningitis, septicaemia and respiratory infections due to group B streptococci in infants and neonates.

It is also suggested that, since infection in neonates[8] is mostly acquired from infected mothers[9] during vaginal passage, a rapid test on vaginal swabs of mothers during the last trimester should be advised and treatment carried out when necessary. Dipstick ELISA has also been developed for group B streptococci.

Enteric and Diarrhoeal Disorders

Each year diarrhoea kills five million children under five years of age in the Third World. WHO recommends that infectious diarrhoea be treated with correct rehydration and continuous feeding. More than 90% of all watery diarrhoea may be managed with oral rehydration, while antibiotics are only used to treat severe shigellosis or cholera.

A number of antigen-detecting tests are available. In cases of food poisoning, the enterotoxins of *Staphylococcus aureus* can be detected by coagglutination[10]. Antibiotic-associated diarrhoeas due to *Clostridium difficile* are fairly common and latex agglutination is available for *Clostridium difficile*[11] antigens. Recently, a monoclonal antibody-based coagglutination format test has been developed for the detection of *Vibrio cholerae* from stools of cholera patients[12].

Group B streptococci are responsible for severe infections such as meningitis and neonatal septicaemia, and rapid detection is essential for early treatment.

During field trials in Guatemala and Bangladesh, this test was found to be as sensitive as culture methods and highly specific. Recently, an epidemic of non-O1 *Vibrio cholerae* O139 has spread widely in the Indian subcontinent, and a similar monoclonal antibody-based coagglutination test has been developed for this strain of *Vibrio cholerae* O139.

Enteric fevers are very common in countries of the Region and their diagnosis at the peripheral level is of great importance. For antigen detection, a semi-culture method for blood, with pathogen identification by the ELISA technique, has been developed by the National Institute of Immunology in India, and this technology has been transferred to manufacturers. The same kit also assesses antibiotic sensitivity, which is essential today since more than 70% of strains of salmonellae have become multidrug-resistant.

A coagglutination test has also been described[13] for *Salmonella typhi* and found to have an 85% sensitivity.

As far as antibody detection is concerned, the Widal test is widely used and a simple slide agglutination test has now become available for salmonella antibodies. Very soon, this test will be adapted for use at the periphery.

Helicobacter pylori is accepted as the causative agent of type B gastritis, peptic ulcer disease and chronic atrophic gastritis, a lesion that is a precursor of gastric carcinoma. The presence of *H. pylori* is strongly associated with chronic, diffuse superficial gastritis of the

Diarrhoea kills five million children under five years of age in the Third World every year.

fundus and antrum. The organism is implicated as a primary causative factor in duodenal ulcer disease, gastric ulcer and non-ulcer dyspepsia.

Serodiagnostic tests permit rapid, non-invasive, sensitive and specific detection of *Helicobacter pylori*. These tests for IgA, IgM and IgG class antibodies are reported to be useful as primary diagnostic tests and obviate the need for endoscopic examinations.

Enzyme immunoassays are expected to be developed in dipstick formats and will be useful for rapid diagnosis of *Helicobacter pylori* infections.

Sexually Transmitted Diseases

Sexually Transmitted Diseases (STDs) are a reason for great concern in most developing countries. The most prevalent bacterial STDs are syphilis, gonorrhoea and chlamydial infections. The emergence of AIDS is a great threat, and STDs with the same risk factors are now linked with AIDS for control purposes. Thus, the diagnosis of STDs, even at the peripheral level, has assumed major significance.

There is no test for the detection of *Treponema pallidum* antigen. A coagglutination test is now available for the detection of *Neisseria gonorrhoeae*[14] antigen in exudates. A dot blot ELISA test has been developed for the detection of chlamydial antigen in urethral exudates and cervical smears[15, 16].

For the diagnosis of syphilis, the main anchor is detection of reaginic

Table 3

Antigen detection in bacterial infections

Condition/Bacterial infection	Sample	Test
Sore throat (*Streptococcus* A)	Throat swab	Latex agglutination, coagglutination, dipstick ELISA, ICA, optical immunoassay
Meningitis (*H. influenzae* b, pneumococci, meningococci, group B streptococci	CSF	Latex agglutination, coagglutination, ICA
Vaginitis (group B streptococci)	Vaginal swab	Dipstick ELISA
Gonorrhoea	Exudate	Latex agglutination, coagglutination
Clostridium difficile A/B	Stool	ELISA, coagglutination
E.coli O157 : H 7	Stool	ELISA
V. cholerae O1 and O139	Stool	Coagglutination
Salmonellosis	Blood	Dipstick ELISA
Tuberculosis	CSF	Latex agglutination, ICA
Chlamydia	Urethral exudate/ genital swab	Dipstick ELISA

antibodies, especially VDRL. The toluidine red unheated serum test (TRUST) is a simple and rapid test and has identical sensitivity and specificity but is much simpler to perform[17].

Mycobacterial Infections

Tuberculosis (TB) and leprosy are common in the South-East Asia Region. Both diseases are chronic and require prolonged chemotherapy, close supervision and follow-up.

At the periphery, it may be necessary to depend on microscopic examination of stained smears for diagnosis, but the bacterial load must be at least 10^5/mL for easy detection by microscopy. Detection of mycobacterial antigen by latex agglutination[18] is being investigated. This may be helpful in paucibacillary phases as well as in CSF from cases of tuberculous meningitis.

Antibody detection techniques by particle agglutination, ELISA and ICA for tuberculosis and particle agglutination for leprosy are now available. Evaluation of their sensitivity, specificity and interference by concurrent HIV infection, prior BCG vaccination and infection by saprophytic mycobacteria is needed before they could be introduced at the peripheral level.

Leptospirosis

A dot ELISA assay has been described recently to detect IgM antibodies against leptospiral species. The test has proved to be effective for diagnosis of human leptospirosis in the acute stage. In this IgM assay the sonicated extracts from cultures of serovar, braziliensis, canicola, cynopteri, haptomadis and icterohaemorrhagieae were laid down as antigen dots on nitrocellulose membrane[19].

Table 4
Antibody detection in bacterial infections

Condition/Bacteria infection	Sample	Test
Typhoid fever	Serum	Slide agglutination
Syphilis	Serum	Slide flocculation, TRUST
Rheumatic fever	Serum	ASO, ASK, Latex agglutination, ICA
Leprosy	Serum	PGL-1 antibody—Particle agglutination
Tuberculosis	Serum	ELISA for detection of IgG and IgA antibodies against antigens of tubercle bacilli
Mycoplasma pneumoniae	Serum	ELISA
Helicobacter pylori	Serum	ELISA, ICA for detection of IgG, IgM and IgA antibody
Leptospirosis	Serum	Dot ELISA

Subsequent procedures were cheap, easy to perform and did not require costly equipment. The positive results were indicated visually by the development of a well defined, purple-blue colour in the dot. The procedure would be of immense use in rapid diagnosis of human leptospirosis in future.

Mycoplasma Infections

Mycoplasma are distinct from true bacteria and viruses as they lack a cell wall and grow in cell-free media. *Mycoplasma pneumoniae* causes atypical pneumonia in children and young adults and is responsible for different neurological, cardiac, gastrointestinal and genitourinary infections. Man is the sole reservoir of infection for *Mycoplasma pneumoniae*. A highly purified lipid antigen of *Mycoplasma pneumoniae* is employed in the quantification of IgG or IgM antibody[20]. A self-contained kit for mycoplasma identification, which gives results within 24 hours, has recently been released. The antibiotic sensitivity pattern is also indicated simultaneously.

Mycoplasma pneumoniae causes atypical pneumonia in children and young adults and is responsible for different neurological, cardiac, gastrointestinal and genitourinary infections.

A simple 10-minute assay to detect IgM antibody has been useful in laboratories assaying small numbers of samples. The test, an ELISA format, does not involve IgM separation or IgG removal. The test is appropriate for use in laboratories for rapid testing of patients with primary atypical pneumonia[21]. Tables 3 and 4 summarize the tests that should be available for bacterial infections.

5. Rapid Diagnostic Tests for Mycotic Infections

SUPERFICIAL MYCOSES are very common in the countries of South-East Asia, more so in areas with humid tropical climates. Diagnosis of these diseases depends mostly on microscopic examination of scrapings from lesions, and may need referral to intermediate level laboratories. There are no simple rapid diagnostic kits available as yet for superficial mycoses (Tables 5 and 6).

Invasive mycotic diseases are being encountered more and more in the countries of South-East Asia. Some fungi causing opportunistic infections have recently become more aggressive due to the increasing incidence of AIDS. Antigenic detection of *Cryptococcus*[22] in CSF and serum is now possible with the latex agglutination format. *Candida* infections are mainly found in immunocompromised patients who are receiving intensive chemotherapy, and both ELISA[23] and latex agglutination[24] tests have been developed for antigen detection. Patients with invasive candidiasis are rarely detected because the antigen is complexed as immune complexes and may evade detection

Some fungi causing opportunistic infections have recently become more aggressive due to the increasing incidence of AIDS.

Table 5

Antigen detection in mycotic infections

Organism	Sample	Test
Cryptococcus	CSF, serum	Latex agglutination, ELISA
Candida	Serum	Latex agglutination
Aspergillus sp.	Serum	Latex agglutination
Penicillium marneffi	Serum	Latex agglutination

Table 6

Antibody detection in mycotic infections

Organism	Sample	Test
Aspergillus sp.	Serum	IgA ELISA, IgM ELISA
Aspergillus fumigatus	Serum	IgG ELISA
Candida albicans	Serum	IgG/IgM/IgA ELISA

by latex agglutination. An ELISA test has also been developed for the detection of antigen in the serum of patients with disseminated aspergillosis[25].

Serological tests have been developed for the main opportunistic invasive fungal infections, such as candidal mycoses and aspergilloses.

Serological tests are frequently used to ascertain the clinical significance of *Candida* species isolates. The detection of IgA and IgM antibodies indicate a fresh infection. Rising titres of IgG antibodies to *Candida albicans* antigen may reflect invasive candidiasis in an immunocompetent patient. Immunosuppressed patients often fail to produce antibodies so a negative antibody test does not rule out the disease. An antigen detection test is recommended in these cases.

Infection with the dimorphic fungus, *Penicillium marneffi* was reported recently in Thailand in patients with AIDS. An early diagnosis of disseminated penicilliosis was possible with a latex agglutination test to detect antigenaemia[26].

6. Rapid Diagnostic Tests for Parasitic Infections

THE COMMON PROTOZOAL and helminthic diseases rampant in the countries of the South-East Asia Region are malaria, amoebiasis, filariasis and helminthiasis. With the advent of AIDS in the Region, detection of opportunistic infections due to *Cryptosporidium* and *Pneumocystis carinii* are an important diagnostic necessity. Control programmes of major parasitic diseases rely almost exclusively on microscopic diagnosis. However, certain diseases such as leishmaniasis, extraintestinal amoebiasis, echinococcosis and toxoplasmosis, need laboratory tests for diagnosis.

Malaria

Malaria is endemic in most countries of the South-East Asia Region. Increasing resistance of *Plasmodium falciparum* to chloroquine is a worrying development. Since almost all malarial deaths are due to *Plasmodium falciparum*, early diagnosis of infection is of great

Malaria is endemic in most countries of the South-East Asia Region. Increasing resistance of *Plasmodium falciparum* to chloroquine is a worrying development.

importance in the treatment of the infection and control of the disease.

Conventional diagnosis based on the thick smear technique takes a long time in peripheral areas. However, a simple dipstick test has now been developed based on detection of histidine-rich protein (HRP-2) released from erythrocytes parasitized by *P. falciparum*. Monoclonal antibodies against HRP-2, fixed on a test strip, react with haemolysed blood samples from positive patients. The test takes three minutes and can be easily conducted by technicians at primary health centres.

The test has 97 per cent sensitivity and 86 per cent specificity. In a few cases there may be cross-reaction with *P. vivax* but not with other malarial or blood parasites. An ICA has been standardized to detect *Plasmodium falciparum* infection in whole blood using two antibodies specific for HRP-2. Preliminary investigations indicate ICA to be a simple and practical method for rapid diagnosis of falciparum malaria at the PHC level[27].

Malaria, amoebiasis, filariasis and helminthiasis are common parasitic diseases in the South-East Asia Region.

Amoebiasis

Amoebiasis is also widespread in countries of the South-East Asia Region. Intestinal amoebiasis is usually diagnosed microscopically, but for extraintestinal amoebiasis, dipstick ELISA tests for antibody detection have been developed.

Dipstick ELISA tests for antigen detection in stools and in liver exudates[28] are also available.

Toxoplasmosis

Toxoplasmosis is a widespread infection in both humans and animals and is a significant cause of mortality and congenital malformation when the disease is acquired *in utero*.

A dot immunoblot technique has been developed to detect *Toxoplasma gondii* antigens in serum and CSF, which has a sensitivity of 40 to 130 pg[29].

An ELISA test has also been developed for the detection of antibodies[30].

Helminthic Infections

Helminthic infections are widespread in the Region. The majority of such infections can be detected microscopically, although a dipstick ELISA test has been developed for both cysticercosis[31] and dracunculiasis.

Leishmaniasis

Leishmaniasis is mainly found in the eastern parts of India and has spread to Nepal, Bhutan, Bangladesh and Myanmar. The main strategy for detection has been serological testing for increased gamma globulins. However, a dipstick ELISA has now been developed for detecting the antibody of *Leishmania donovani*[32], making the diagnosis more specific.

A co-infection with HIV could be associated with no antileishmanial antibody. This could vitiate the sensitivity and specificity of antibody tests for *Leishmania donovani*.

Giardiasis

Giardia lamblia, the causative organism of giardiasis, is responsible for extensive diarrhoea and vomiting, malabsorption syndrome, epigastric pain and cholecysto-

Table 7

Antigen detection in parasitic diseases

Disease/Organism	Sample	Test
Malaria (*P. falciparum*)	Blood	Dipstick ELISA, ICA
Amoebiasis	Stool, liver exudate	ELISA
Cryptosporidiosis	Stool	ELISA
Giardiasis	Stool	ELISA

Table 8

Antibody detection in parasitic infections

Disease	Sample	Test
Amoebiasis	Serum	ELISA, latex agglutination, indirect haemagglutination
Toxoplasmosis	Serum	ELISA
Kala azar	Serum	ELISA
Cysticercosis	Serum	ELISA
Dracunculiasis	Serum	Dipstick ELISA
Echinococcosis	Serum	ELISA

pathies in South-East Asia. Transmission is by ingestion of cysts in contaminated water or food infected by flies. Diagnosis is effected by demonstration of cysts in stools or trophozoites in duodenal aspirate.

An ELISA format has now been standardized to detect cysts and trophozoite antigens in stool samples[33]. This technique, unlike microscopy, does not require the presence of intact organisms in the specimen. The kit is meant to enhance microscopic examination and is practicable in the following situations:

- Screening family members of a patient with giardiasis.
- Screening large populations, such as food handlers.
- Examining paediatric populations to rule out *Giardia* infection.
- As a less expensive alternative to microscopy for community screening.

With this technique, there is no need for concentration of stool samples. Tables 7 and 8 summarize the tests available for detection of parasitic infections.

7. Rapid Diagnostic Tests for Viral Infections

UNTIL THE 1970s, diagnosis of viral infections was not a practical proposition at the PHC level. Specific diagnosis was possible only in laboratories with facilities for tissue culture and required special equipment as well as well-trained staff, which was very costly. The procedures were time-consuming and there were no antiviral drugs for therapy.

However, the development of antiviral chemotherapy, including drugs such as acyclovir, ganciclovir, rimantadine, interferons, zidovudine, zalcitabine, ritonavir, indinavir, azidothymidine and ribavirin, boosted the generation of innovations in rapid diagnostic procedures for viral infections.

Following the standardization of systems using the ELISA, fluorescent antibody and radio-immunoassay tests during the 1980s, self-contained kits have been marketed for use in developed countries.

With the development of self-contained kits, diagnosis of viral infections or quantification of antiviral antibodies has become a reality even in developing countries that lack sophisticated facilities for cell culture. There has been sudden interest in simplification to ensure single-step test procedures that can be completed in one day.

Rapid test systems that employ a card, a stick or a strip for impregnation with blood or saliva from patients/prospective blood donors have recently become available. Results are obtained within one to three hours.

Hepatitis

At least five viruses that cause hepatitis in humans are known: hepatitis A and hepatitis E (Non-A Non-B enteric) are spread enterically, while hepatitis B, hepatitis D and hepatitis C (Non-A Non-B bloodborne) are bloodborne.

Hepatitis B (HBV) virus infection is a worldwide problem. Some 280 million people are chronically infected with hepatitis B virus. More than three-quarters of the world's population is living in areas where the prevalence of chronic HBV infection is two per cent or more.

Hepatitis B virus (HBV) infection is a worldwide problem chronically infecting 280 million people.

Chronic carriers of HBV have an added risk of developing primary liver cancer, and almost 25 per cent of chronic carriers die of primary liver cancer or cirrhosis as adults. Screening for hepatitis B surface antigen (HBsAg) among prospective blood donors would prevent virus transmission through blood transfusion, while immunization of babies born to HBsAg-carrier mothers would further reduce the occurrence of HBsAg in populations.

A simplified latex agglutination test for HBsAg detection is available and has a sensitivity of one ng per mL. A promising ELISA, with a sensitivity of 0.1 ng per mL is available and is suitable for use at the periphery and in blood banks[34]. The recent introduction of ICA technology to detect HBsAg appears very exciting. Anti-HBs (hepatitis B surface)[35] antibodies appear in the later stage of infection and provide protection against recurrence of infection.

Anti-HBs assays are conducted using sensitized chicken erythrocytes by a procedure that is simple, requires no special equipment or apparatus and produces results within one to two hours at room temperature. These assays provide valuable diagnostic or prognostic information regarding individuals and are also helpful in epidemiological studies on virus transmission. An ELISA format test is also available.

Bloodborne hepatitis C is also an emerging problem in South-East Asian countries, and has the same clinical and epidemiological features as hepatitis B. An antigen capture ELISA test is available for HBV, while an ELISA dipstick test has recently become available which can detect HCV antibody in serum as well as in urine.

Bloodborne hepatitis C is also an emerging problem in South-East Asian countries, and has the same clinical and epidemiological features as hepatitis B.

Waterborne hepatitis infections are caused by hepatitis A and hepatitis E viruses. For the detection of hepatitis A infection, an ELISA test with a 100 per cent sensitivity for anti-hepatitis A virus IgM in serum has been developed. This is a very good diagnostic as well as prognostic tool. Diagnostic methods based on HEV antibody detection include an ELISA test and are now available.

Gastrointestinal Viruses

Rotaviruses are responsible for diarrhoea in infants and young children and for gastroenteritis in adults. Specific diagnosis of rotavirus in clinical materials would help prevent unnecessary use of antibiotics and ensure judicious management of gastroenteritis. Latex agglutination[36] and ELISA kits[37] are available for the detection of rotaviruses. Though cheaper, the latex agglutination test is not as sensitive as the ELISA test, and yet it may be useful in peripheral laboratories till cheaper dipstick ELISA tests become available.

Diagnosis of rotavirus in clinical materials would help prevent unnecessary usage of antibiotics and ensure judicious management of gastroenteritis.

Respiratory Viruses

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is the single most common cause of lower respiratory tract infection in infants and young children. During winter, epidemics of bronchiolitis occur in children. Specific viral diagnosis based on the antigens present in the clinical material can assist in patient management and control of epidemics.

ELISA tests with 95 per cent sensitivity are available[38]. Since effective antiviral treatment for RSV is now available (amantadine), early treatment of diagnosed patients would be useful.

Influenza virus

Influenza A virus infections have a significant global impact on health. Early identification of influenza outbreaks can enable public health officials to initiate appropriate control measures, such as immunization of high-risk individuals or treatment with antiviral agents.

Rapid diagnosis using clinical material from immunocompromised patients would allow timely antiviral therapy (amantadine or rimantadine) for the treatment or even prevention of influenza A virus infection. An ELISA format test for the detection of influenza A virus is now available[39], but is not very sensitive (50–60 per cent) although it has high specificity.

In this test, antigenic drift is not an issue. Efforts are being made to develop highly sensitive ELISA diagnostic kits.

Measles

The simplified dot immunobinding enzyme immunoassay[40] has potential in screening for seropositivity and quantification of IgG[41] antibodies.

The availability of a rapid test to detect IgM antibodies would enable specific diagnosis in acute phases of the illness with atypical clinical manifestations. An enzyme immunoassay for qualitative and quantitative assay for measles virus-specific IgM has been standardized.

TORCH (Toxoplasma, Rubella, Cytomegalovirus, Herpes) Complex Viruses

Rubella virus

The latex agglutination and enzyme immunoassay tests are effective for detection of rubella antibody[42].

Early identification of influenza outbreaks can enable public health officials to initiate appropriate control measures.

Even though rubella infections are mild, causing skin rashes and enlargement of cervical lymph nodes without any permanent damage, infections in women during the first trimester of pregnancy can lead to heart malformations, cataracts and mental defects in the foetuses. The demonstration of rubella antibody in a pregnant female enables appropriate management of the pregnancy.

Cytomegalovirus

Cytomegalovirus (CMV) infections may be asymptomatic or associated with mild influenza-like disease. Some people may develop fever, muscle pain, and enlargement of the liver and spleen with generalized lymph node enlargement. CMV infections are occasionally associated with pneumonia or neurological disorders.

In immunocompromised hosts, including premature infants, patients being treated for cancer or receiving immunosuppressive drugs, or recipients of bone marrow or organ transplants, infections are very severe and even life-threatening. Simplified latex agglutination[43] and enzyme immunoassay techniques[44] are available for determining the CMV immune susceptibility of a patient.

Herpes simplex virus

Herpes simplex virus (HSV) is responsible for localized lesions, such as herpes labialis, stomatitis, keratoconjunctivitis, and infections of the genital tract. HSV can also cause life-threatening infections such as pneumonia, meningo-encephalitis, and systemic infections in the newborn. The use of ELISA in the diagnosis of HSV infections[45] enables the use of specific antiviral drugs, such as acyclovir, famciclovir, ganciclovir and foscarnet.

The use of ELISA in the diagnosis of HSV infections enables the use of specific antiviral drugs, such as acyclovir, famciclovir, ganciclovir and foscarnet.

The coagglutination test is also available but is of lower sensitivity than ELISA. It is important to test for HSV antigen in vaginal swabs of expectant mothers, and, when positive, to plan caesarean sections to avoid infections of the newborn from the vaginal passage during birth.

Varicella–Zoster

Varicella–zoster coated latex particles[46] are useful for screening sera for anti-varicella–zoster antibodies, and the simplified assay is useful in testing susceptible persons for their immunity to the virus. The test is useful in screening recipients of antiviral drugs, as both qualitative and quantitative testing is possible.

Dengue Virus

Dengue fever is transmitted by mosquitos and can be due to any of four serotypes of dengue virus. It causes high fever, severe pain in muscles, joints and bones and can be accompanied by skin rashes. A dot blot enzyme immunoassay[47] format is useful for detecting antibodies to types 1, 2, 3 and 4 dengue viruses.

A simple dot blot dipstick (DS) using graded concentrations of sucrose gradient purified dengue antigens on a nitrocellulose paralleling four-fold strip has recently been standardized to detect anti-dengue antibodies.

It is possible to measure both IgG and IgM antibodies using whole blood, serum or plasma. The utility of DS in distinguishing a primary and secondary dengue infection is being evaluated.

Epstein–Barr virus

Infectious mononucleosis (IM) is usually a self-limiting disease caused by Epstein–Barr virus (EB). The common symptoms are fatigue, pharyngitis, fever, lymphadenitis, splenomegaly and hepatitis. In close populations in developing countries, most children become infected by three years of age and symptoms are mild. During the acute phase of the illness, heterophil antibodies appear and are primarily of the IgM class.

Latex agglutination or enzyme immunoassay[48] techniques employ goat anti-human IgM antibodies which enable reliable diagnosis without the use of bovine, horse or sheep erythrocytes.

Parvovirus

Parvovirus B19 is the aetiological agent of erythema infectiosum, transient aplastic crisis in patients with chronic haemolytic anaemia, acute arthritis in adults and persistent anaemia in immunocompromised patients. B19 has also been implicated as a cause of hydrops foetalis and foetal loss during pregnancy.

ELISA using recombinant antigens is useful in detection of parvovirus-specific IgG and IgM antibodies. A serological diagnosis of arthralgia, polyarthritis, exanthems and lymphadenopathy is possible[49]. Moreover, saliva may be a convenient alternative to serum for diagnosis of a recent B19 infection.

Human Immunodeficiency Virus (HIV)

The last decade witnessed the worldwide spread of acquired immunodeficiency syndrome (AIDS). The prevalence of AIDS in South Asian countries has increased rapidly. HIV is the causative organism of AIDS, and belongs to the family of retroviruses. Retroviruses are RNA viruses that produce virus DNA using the enzyme reverse transcriptase. This virus DNA becomes integrated with the genome of T4 helper lymphocytes, leading to chronic infection.

Sensitivity and specificity are two major factors that determine the accuracy of a test.

Serological tests for anti-HIV antibody are the basis for diagnosis of HIV infection. Antigen detection has not yet been standardized. Antibody to HIV appears in the blood within three months of initial infection in 95 per cent of persons, while by six months, over 99 per cent of HIV-infected persons become positive.

Several different types of laboratory tests for detecting HIV antibody in human serum are available, and have high sensitivity and specificity. The objectives of HIV antibody testing are screening of blood and blood products for transfusion safety, diagnosis of HIV infection and surveillance of the disease.

Sensitivity and specificity are two major factors that determine the accuracy of a test in distinguishing between infected and uninfected persons. A test with a high sensitivity will have few false-negative results, and therefore only tests of the highest possible sensitivity should be used when there is a need to minimize the rate of false-negative results (e.g. in transfusion/donation safety).

A test with a high specificity will have few false-positive results and should be used when there is a need to minimize the rate of false-positive results (e.g. in diagnosis of HIV infection in an individual).

Strategies for HIV Antibody Testing

WHO recommends three testing strategies to maximize accuracy while minimizing cost[50]. Which strategy is most appropriate will depend on the objectives of the test and the prevalence of HIV in the population.

Strategy I

All sera are tested using an ELISA test or another rapid/simple assay process. Serum that is reactive is considered to be HIV antibody positive. Serum that is non-reactive is considered to be HIV antibody negative.

Strategy II

All sera are first tested with an

Table 9

Antigen detection in viral infections

Disease/Organism/Antigen	Sample	Test
Hepatitis B surface antigen	Serum, saliva	Latex agglutination, RPHA, dipstick ELISA, ICA
Rotavirus	Stool	Dipstick ELISA, latex agglutination
Influenza virus	Throat washings	ELISA
Respiratory syncitial virus	Throat washings	ELISA
Herpes simplex	Throat washings, genital swab, urine	Coagglutination
Adenovirus	Stool	ELISA

ELISA or another rapid/simple assay system. Any serum found reactive on the first assay is retested using a second ELISA test or rapid/simple assay method based on a different antigen preparation and/or different test principle (e.g. indirect versus competitive).

Serum that is reactive to both tests is considered to be HIV antibody positive. Serum that is non-reactive with the first test is considered to be HIV antibody negative. Any serum that is reactive on the first test but non-reactive on the second test is also considered to be antibody negative.

Table 10

Antibody detection in viral infections

Disease/Organism/Antigen	Sample	Test
HIV	Serum, saliva	Particle agglutination, dipstick ELISA, cassette device, ELISA, ICA
HAV-IgM	Serum	Dipstick ELISA
Anti-HBs	Serum	RPHA, ICA, ELISA
HEV	Serum	ELISA
HCV	Serum, urine	Dipstick ELISA, ICA
Cytomegalovirus	Serum	Latex agglutination
Varicella–Zoster	Serum	Latex agglutination
Measles IgM, IgG	Serum	ELISA, dipstick ELISA
Dengue	Serum	ELISA, dipstick ELISA
Heterophil antibody	Serum	ELISA, ICA, latex agglutination
Infectious mononucleosis	Serum	ELISA
Herpes simplex IgM	Serum	ELISA
Parvovirus IgM, IgG	Serum	ELISA
Mumps IgM, IgG	Serum	ELISA
Respiratory syncitial virus	Serum	ELISA
Hantavirus	Serum	Strip immunoblot

Strategy III

As in strategy II, all sera are first tested with an ELISA or rapid/simple assay system, and any reactive samples are retested using a different assay procedure. Strategy III, however, requires a third test for sera found reactive in the second assay. The three tests in this strategy should be based on different antigen preparations and/or different test principles. Serum reactive in all three tests is considered to be HIV antibody positive. Serum that is non-reactive on the first test is considered to be HIV antibody negative as is serum that is reactive in the first test but non-reactive in the second. Serum that is reactive in the first and second tests but non-reactive in the third test is considered to be equivocal and should be retested after four weeks.

When selecting HIV antibody tests for use in strategies II and III, the first test should have the highest sensitivity, whereas the second and third tests should have higher specificities than the first.

For surveillance purposes WHO recommends strategy I where HIV prevalence is greater than 10 per cent, and strategy II where prevalence is at or below 10 per cent.

When the objective of testing is diagnosis of asymptomatic individuals, the WHO Global Programme on AIDS (GPA) recommends strategy II in areas where HIV prevalence is above 10 per cent, and strategy III in areas where prevalence is at or below 10 per cent.

When the objective of testing is diagnosis of individuals with signs and symptoms indicating possible HIV infection, strategy II is recommended. WHO recommendations for surveillance purposes are strategy I where HIV prevalence is greater than 10 per cent, and strategy II where prevalence is at or below 10 per cent.

Very simple dipstick ELISA format tests of high sensitivity and specificity are now available and can be used at the periphery. Particle agglutination (Serodia) is highly sensitive and is used as a screening test in peripheral areas.

GPA recommends strategy I when the objective of testing is to screen blood for transfusion safety. If a blood donor is to be notified of the test result, as is done in some blood screening programmes, then the testing strategies for patient diagnosis must be used.

Tables 9 and 10 summarize tests available for viral infections.

8 Monitoring Vaccine Response Among Recipients

IN THE Expanded Programme on Immunization (EPI), the problem of non-responders is being faced in many areas of the world. Vaccine-induced protection in individuals is examined using complicated test procedures involving cell cultures, animals and haemagglutination inhibition utilizing mouse or rat erythrocytes. Simplified procedures that can assist in locating vaccine-induced responses in individuals following prophylactic or therapeutic administration of vaccine preparations are gradually being introduced.

Wide availability of such procedures would help in determining the immune responses of individuals and in carrying out

epidemiological investigations on the effects of particular vaccines. Rapid assay procedures are standardized for vaccines included in the WHO (EPI), and also for other vaccines.

Simplified procedures that can assist in locating vaccine-induced responses in individuals following prophylactic or therapeutic administration of vaccine preparations are gradually being introduced.

Rapid Tests for Seroconversion to EPI Vaccines

Pertussis

A precise method has been described for estimating antibody levels to *Bordetella pertussis* using polystyrene balls in an ELISA format[51]. Antibodies against pertussis toxin and filamentous haemagglutinin can be measured within three hours, and the results clearly differentiate between antibody positivity and antibody negativity in individuals.

Tetanus

Classically, neutralizing antibodies to tetanus have been measured using the mouse neutralization test. As an alternative to this test, an immunoassay involving inhibition of toxin-binding capacity in human sera has been described[52]. Preliminary results of this test are encouraging and show good correlation with results from mouse neutralization tests.

A simple and quick procedure that uses sensitized erythrocytes, allows titration of antibodies in emergency care units would be helpful in seroepidemiological surveys of post-vaccinal immunity and evaluation of immune status of the population. Furthermore, it is also possible to express antitoxin titres in international units.

Diphtheria

Preliminary efforts to replace animal testing with an ELISA format to measure antitoxin have not been as rewarding[53] as in the case of tetanus antitoxin. Nevertheless, it is likely that appropriate technological modifications will ensure the availability of a simplified test for anti-diphtheria antitoxin measurements in the near future.

Measles

Recent reports on the dot-immunobinding assay, which is a modified immunoassay, indicate that it has potential for use in areas with minimum laboratory facilities.

Rapid Tests for Response to other Vaccines

Hepatitis B vaccine

Antibody production following plasma-derived or recombinant vaccination for hepatitis B virus can easily be estimated using the passive haemagglutination and ELISA tests. Estimations of antibody to hepatitis B surface antigen (anti-HBs) in the field would greatly assist control programmes for hepatitis B virus in endemic areas.

Rabies vaccine

Animal test procedures for rabies antibodies were complicated and gave results only after two to three weeks. It is now possible to sensitize latex particles[55] with purified rabies virus particles. The sensitized particles rapidly agglutinate in the presence of antibodies. However, this test detects seroconversion only if the antibody level is more than 2.5 IU/mL.

Rubella vaccine

A latex agglutination[42] format test for anti-rubella IgG class antibody is available and is valuable in quantifying post-immunization responses to rubella vaccines. Apart from qualitative testing, this simplified testing procedure also allows antibody titres to be quantified locally.

Rapid Tests for Urine

DIPSTICK TECHNOLOGY for estimating chemicals in urine is widely available. Multianalyte strips for assessing various parameters in urine are also available, but except for a bianalyte strip for urinary albumin and glucose, these may be of value only in advanced laboratories. The nitrocellulose strip is impregnated with specific chemical reagents and on dipping it in urine, instant colour change can denote qualitative and quantitative values of various chemical parameters.

It is possible to test for specific gravity, pH, glucose, ketones, proteins, bilirubin, urobilinogen, nitrites and blood. The colour changes are compared with a master chart.

The master chart incorporates the colours expected following reactions with individual urinary components. Apart from a result as 'positive' or 'negative' for any component in urine, it is also possible to obtain a rough idea about the quantity of the pathological component. For example, one can get an idea about the quantity of glucose in a urine sample on visualization of the colour intensity in the glucose component of the strip. Some manufacturers provide charts for this purpose. The strips can be used in remote areas, do not require a heat source and do not involve urine filtration or additional chemicals.

Blood Glucose Estimation

A large number of patients with diabetes mellitus have raised blood glucose levels which must be estimated frequently to determine the response to anti-diabetes therapy and dietary measures.

A simplified test procedure was developed in the 1970s. This procedure does not require venous blood and glucose levels are estimated from samples drawn by the finger prick method. The blood reacts directly with chemicals impregnated on a strip, and the reaction is completed within a few minutes, the end-point being the development of coloured products. The colour developed allows estimation of blood glucose levels, and the intensity of colour is measured colorimetrically in a glucometer.

This apparatus is handy and compact and can be used in remote areas. The results give a fair idea of the state of illness or response to treatment and can help in mass screening for diabetes mellitus in a short time period (Table 11).

Table 11

Rapid tests available for clinical chemistry

Uni-, bi- or multianalytic strips	
Urine	Glucose, Protein
	pH, Specific gravity
	Bilirubin, Urobilinogen
	Ketones, Nitrite, Blood
Blood	Glucose

Automated Procedures for Clinical Chemistry

A promising technique is available for the quantitative estimation of various clinical chemistry values in blood, plasma or serum. The procedure involves the use of a compact reflectance photometer with fully automatic evaluation. Temperature regulation, calibration, evaluation and calculation are all under the control of a microprocessor.

The apparatus, marketed by Boehringer Mannheim GmbH (Reflotron^{R}), works at room

temperature and has a printer attachment. Using 30 µL of serum, plasma or whole blood, it is possible to carry out tests for glucose, haemoglobin, urea, creatinine, uric acid, cholesterol, triglycerides, amylase, creatine kinase, potassium, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and high density lipoprotein (HDL) cholesterol. The technique is simple, reliable, rapid and feasible at all levels.

The present system works at 110–120 volts and delivers 30 µL of blood at fixed spots on paper strips specific for each test. The paper strips are inserted in the apparatus which carries out all steps in the testing procedure and issues the final results on the screen or through the printer.

Multianalyte portable test systems could be used for frequent estimations in routine and emergency clinical chemistry investigations.

The tests are carried out on undiluted samples and, with appropriate modifications by the manufacturer, it is possible to use the apparatus in areas with irregular power supply or no power supply at all. Considering the initial and running costs, this system should be used only in advanced laboratories for the time being.

The contribution made by frequent estimation of urea, cholesterol, proteins, electrolytes and enzymes[56] towards clinical care is immense. As opposed to conventional and complicated assay systems, multianalyte portable test systems involve direct application of specimens onto slides or sticks, and results can be observed in the mini-computer assembly of the appliance.

Though the initial cost is high, such procedures appear to be promising for use in countries of the Region in routine and emergency clinical chemistry investigations. Simplified enzyme immunoassay formats are being standardized to assay hormone and drug levels with the naked eye.

10. Miscellaneous Rapid Diagnostic Tests in Clinical Pathology/Haematology

HEALTH LABORATORIES at the peripheral level are expected to conduct tests for diagnosis of disease and for public health activities (such as communicable diseases). Some simple procedures have been designed for pregnancy, CRP and rheumatoid factor (RF) testing.

Pregnancy Testing

Testing for pregnancy was simplified in 1970 by estimation of human chorionic gonadotrophin (hCG) in urine. Today, very simple tests are available which can be carried out even at the peripheral level. Latex agglutination[57] and dipstick ELISA tests are commonly available. Latex agglutination tests are quite sensitive and very cheap.

C-Reactive Protein (CRP)

This protein appears in the blood stream as an indication of acute inflammatory disease and is more sensitive than the erythrocyte sedimentation rate (ESR) in acute phases. C-reactive protein is also of prognostic value. Latex agglutination tests, which can be easily performed, are available for testing for CRP.

Rheumatoid Factor (RF)

This factor appears in the blood of patients with rheumatoid arthritis,

and can easily be detected using a latex agglutination test.

Sickle Cell Disease/Trait

This is a hereditary disease caused by a recessive gene that leads to the production of haemoglobin S and the consequent production of sickle-shaped erythrocytes. Homozygous individuals die early due to severe anaemia caused by premature destruction of the sickle-shaped erythrocytes, while heterozygous individuals have mild anaemia and act as carriers of genetic material for haemoglobin S.

A monoclonal antibody-based assay was introduced to detect haemoglobin S (HbS) in whole blood or blood spots on filter paper. The reagents have long shelf-lives and are supplied in premixed form requiring no reconstitution. The use of monoclonal antibody against adult haemoglobin (HbA) in an identical test format is helpful for differentiating homozygous (SS) and heterozygous (SA) individuals at birth.

Simple and reliable methods for screening of thalassaemia, another genetic red cell defect, at PHC level are being developed and standardized.

Coagulation Profiles

Apart from the conventional bleeding and clotting time assays to monitor coagulation profiles at PHC laboratories, simplified procedures have been introduced for bedside estimation of both extrinsic and intrinsic pathways of coagulation. With the introduction of battery-operated coagulation instruments, estimates of prothrombin time and activated partial thromboplastin time using small volumes of whole blood are feasible by personnel with minimal training.

Estimates of prothrombin time are useful for monitoring extrinsic pathways of coagulation and oral anticoagulant therapy in out-patient clinics. In addition, assays of activated partial thromboplastin time assist in monitoring intrinsic pathways of coagulation and anticoagulation therapy with heparin. Laboratories at different levels should be expected to assist during vascular surgery, haemo-dialysis, haemofiltration and management of thrombophlebitis and thromboembolism.

The bladder tumour antigen (BTA) ICA is superior to cytology in the early detection of bladder cancer.

Tumour Markers

The activity of growing cells in different malignancies is monitored by assaying carcinoembryonic antigen (CEA), alphafetoprotein (AFP) and tissue polypeptide specific antigen (TPS) in tissue sections and blood[58].

The recent availability of a monoclonal immunoassay to estimate TPS levels is promising. A single-step, five-minute ICA is available for detection of bladder tumour antigen (BTA) associated with cancer of the urinary bladder. Preliminary results indicate that BTA ICA is superior to cytology in the early detection of patients suspected of having bladder cancer.

The availability of a dipstick ELISA format test in field laboratories for assaying TPS and other markers would be invaluable in monitoring the growth of malignant tumours as well as their responses to therapeutic measures.

However, the role of these tests at the peripheral level is doubtful although they should be utilized in advanced level laboratories.

Autoimmune Diseases

The diagnosis of antibodies that are directed against one's own tissues and are responsible for different autoimmune diseases has been

possible using fluorescence microscopy at limited laboratory establishments.

Detection and titration of thyroglobulin antibody has recently been simplified by using sensitized gelatin particles. Particle carriers sensitized against thyroglobulin extracted and purified from human thyroid tissue appear to be very promising for diagnosis of autoimmune diseases. Apart from thyroglobulin, sensitized gelatin particles are also available for detecting thyroid microsomal antibody.

The cumbersome procedures for identifying anti nuclear antibodies or antibodies to single-stranded DNA (ssDNA) can be utilized in a few laboratories only. The availability of an enzyme immunoassay format to detect and quantify antibodies to double-stranded DNA (dsDNA) would greatly simplify testing procedures. The high titres against dsDNA are almost exclusively associated with systemic lupus erythematosus.

The future availability of a dipstick ELISA towards dsDNA would help peripheral laboratories in undertaking local screening for autoimmune diseases. However, for a long time to come, its use may be limited to advanced level laboratories.

T4 Cell Counts

T4 cell counts[59] may become increasingly important in view of the AIDS epidemic which is gaining momentum. Different lymphocyte subsets are usually enumerated during flow cytometry using combinations of phycoerythrin and fluorescein isothiocyanate-labelled monoclonal antibody preparations.

Flow cytometers are costly, require daily quality control checks and do not offer a practical solution for T4/T8 counting in the field.

Three methods are now available for simple quantification of T4/T8 cells. Of these three tests, the one utilizing cytospheres is the simplest to perform.

All the three tests described are simpler and cheaper than flow cytometry and the results correlate with results from flow cytometry.

Simplified test formats for enumeration of CD4+ cells, along with a similar simple test to quantify HIV RNA in serum, would assist in monitoring an individual's response to a 2–3 drug combination chemotherapy against HIV infection/AIDS with nucleoside analogues and/or protease inhibitors.

Methods available for simple quantification of T4/T8 cells

1. **Cytospheres[60] consist of latex beads coated with CD4 antibody. These are mixed with anticoagulated whole blood after which the red cells are lysed and the bead-coated cells counted using a haemocytometer and a light microscope.**
2. **Dynabeads T4–T8 Quant are magnetic dynabeads coated with CD4 and CD8 monoclonal antibodies. Whole blood is first mixed with dynabeads coated with CD14, which react wth the majority of non-T4–T8 monocytes. These cells are removed using a magnet. The remaining cells are then mixed with dynabeads coated with CD4 and CD8 antibodies, and are subsequently differentially separated and counted under the microscope.**
3. **An ELISA format has been standardized to estimate CD4 cell protein in lysed whole blood. Lysis releases membrane-bound CD4 protein which is assayed in microtitre plates coated with antibody to human CD4 protein.**

Occult Blood in Faeces

Conventionally, a diagnosis of gastrointestinal bleeding was made by demonstrating the pseudo-peroxidase activity of the haematin in the haemoglobin molecule in stool specimens.

A recently introduced simple latex agglutination test detects human haemoglobin and transferrin in faeces[60]. Latex particles are[61] sensitized with antibodies against human haemoglobin and human transferrin. The presence of haemoglobin and/or transferrin leads to an agglutination of the sensitized latex particles. Unlike conventional tests, latex agglutination is not affected by the presence of substances with peroxidase-like activity or vitamin C in stool samples.

11. Surveillance of Emerging Infectious Diseases

CONTROL OF MANY infectious diseases became possible with the pioneering work of Louis Pasteur and Robert Koch and the introduction of the 'germ theory' of disease. Until recently, there was a widespread feeling that the struggle against infectious diseases was almost won. The means of controlling most of them seemed either available or discoverable without undue difficulty.

During the 1970s, the coordinated international efforts of WHO led to the global eradication of smallpox. There was a widespread feeling that infectious diseases were on the point of elimination.

Nevertheless, the euphoria was short-lived and the resurgence of old diseases and emergence of new ones is a challenge to humanity. These diseases are the leading causes of death and continue to cause major public health problems worldwide.

Emerging infectious diseases are diseases that have either appeared in a population for the first time, or have occurred previously but are increasing in incidence or are spreading to new areas.

An infectious disease may be introduced into a new population from the environment, another species, or other humans, and then establish itself and spread within the new population.

Factors that promote either the introduction of the disease agent or its spread will therefore facilitate disease emergence.

Re-emerging infectious diseases are known communicable diseases that were once declining in a population but are now increasing again. Examples of such diseases include tuberculosis, cholera, plague and diphtheria.

Re-emerging infectious diseases are known communicable diseases that were once declining in a population but are now increasing again. Examples of such diseases include tuberculosis, cholera, plague and diphtheria. Antibiotic resistance in many bacterial pathogens has risen considerably, which is complicating therapy.

International Concern on Emerging and Re-emerging Infections

An efficient surveillance system would help in tackling the emergence and re-emergence of communicable diseases. Appropriate laboratory support would provide early warning leading to rapid intervention for the control of epidemics. An International Collaboration for rapid response to emerging and re-emerging infections was launched in 1996 under the coordination of WHO. WHO is supporting the development

Table 12

NEW EMERGING VIRAL DISEASES

Virus	Place of Isolation/Prevalence	Year
Human immunodeficiency virus		1983 onwards
Rotavirus		1973 onwards
Hepatitis C virus		1989
Hepatitis G		1996
Influenza virus H5N1	Hong Kong	1997
Monkeypox virus	Zaire	1996–1997
Filoviruses		
Marburg	Germany	1969
Ebola	Zaire	1976, 1995
	Sudan	1976,1979
Ebola-like	USA	1989 in monkeys
Ebola-like	Ivory Coast	1990
(non-fatal in laboratory workers)		
Arenaviruses		
Junin	Argentina	1950s
Lassa	Nigeria	1970s
Guanarito	Venezuela	1989
Sabia	Brazil	1990
	USA	1994
(non-fatal in laboratory workers)		
Hantaviruses Renal	Korea, China	1950s
Pulmonary	USA	1993

NEW EMERGING PRION DISEASE

Virus	Place of Isolation/Prevalence	Year
New variant of Creutzfeld–Jakob disease (vCJD)	UK	1990s

NEW EMERGING BACTERIAL DISEASES

Bacterium	Disease	Year of first emergence
Legionella pneumophila	Legionnaire's Disease	1977
Escherichia coli O157: H 7	Haemolytic Uraemic Syndrome	1982
Borrelia burgdorferi	Lyme's Disease	1982
Helicobacter pylori	Peptic Ulcer Infection	1983
H. influenzae, H. aegypticus	Brazilian Purpuric Fever	1987
Vibrio cholerae O139	Cholera	1992

RE-EMERGENT VIRAL INFECTIONS

Arboviruses		
Dengue fever	South-East Asia westwards	1990s
Rift valley fever	Egypt	1970, 1990s
	Mauritania	1987
	Kenya	1997
Yellow fever	Kenya	1990s

of International Collaboration with the help of Member States. The aim is to establish a global network of laboratories in support of public health services.

Rapid detection of epidemics through an efficient and viable surveillance system can facilitate rapid responses. In a poor health environment, emerging and re-emerging infectious diseases may not be detected until they become major threats to the population and cannot be contained with national resources.

The existing public health laboratories are often poorly equipped and unable to diagnose common diseases. National infrastructure must be developed for surveillance systems and health laboratory services must be strengthened to address these problems.

Since 1996, WHO is coordinating an international effort to detect and control communicable diseases through an efficient surveillance system and upgraded diagnostic capabilities. A global network of epidemiological surveillance and infectious disease laboratories has been proposed. It will work in close cooperation with the central coordinating agency—WHO, and with local health authorities and international organizations. An important component in this infrastructure would be the improvement of public health infrastructure and enhancement of expertise and linkages. National laboratories are being identified and will soon be linked electronically to handle such diseases.

Role of Laboratories

Reliable laboratory support is essential in investigation of disease. Isolation, identification and characterization of the pathogen using epidemiological marker typing methods form an integral part of the disease surveillance system. Appropriate laboratory infrastructure—buildings, space, equipment, reagents, manpower development and training programmes—is essential. Well-established linkages with laboratories designated for advanced studies, electronic linkages with information databases and epidemic surveillance systems are vital. Rapid advances in scientific methodology have led to the development of diagnostic reagents for most of the emerging and re-emerging infections. Such tests will soon become available for global use with special attention to their use at the periphery.

RE-EMERGING BACTERIAL DISEASES (1990s)

Disease	Countries
Plague	India
Cholera	Latin America
Diphtheria	Russia
Anthrax	Cuba
Pertussis	North America
Streptococcal infections	North America and Europe
Tuberculosis	Africa and worldwide in association with AIDS
Meningitis	Africa

MULTIDRUG RESISTANCE (1990s)

Organism/Disease	Countries
Shigella dysenteriae	Burundi, Rwanda
M. tuberculosis	Africa and Asia
Pneumococcus	USA and Europe
Enterococcus	USA
Salmonella	Global
Malaria	Global

Table 13

Availability of diagnostic reagents for emerging and re-emerging infections

VIRAL INFECTIONS	
New emerging	
Ebola	ELISA
Lassa	ELISA, IFA
Hantavirus	ELISA
Rift valley fever	ELISA
HIV	Dot ELISA, Dipstick ELISA, Particle agglutination
Hepatitis C	Dot ELISA
Rotavirus	ELISA
Re-emerging	
Dengue fever	Dot ELISA
Yellow fever	ELISA
Hepatitis B	Dot ELISA, Latex agglutination
Measles	IgM and IgG ELISA
Rubella	ELISA
Varicella	Latex agglutination
Herpes simplex	ELISA

BACTERIAL INFECTIONS	
New emerging	
V. cholerae O139	Coagglutination
Lyme's disease	ELISA, IFA
E. coli O157	Cassette device ELISA
Helicobacter pylori	ELISA
Re-emerging	
Plague	ELISA, IFA, Latex agglutination
Tuberculosis	Dipstick ELISA
Streptococcus A infections	Dipstick ELISA

PARASITIC	
Malaria	Dipstick ELISA

12. Feasibility of Provision of Rapid Diagnostics in the South-East Asia Region

IN THIS SECTION, an effort has been made to present the rapid diagnostic kits that are available in the market. These techniques cannot replace conventional ones, but the advantage of early diagnosis and consequent initiation of therapeutic and control measures offsets the disadvantage of not having a complete characterization, antibiogram and epidemiological features of a pathogen.

There is considerable correlation between the sensitivity and specificity of rapid diagnostic techniques and that of conventional testing procedures. In certain instances, such as HIV antibody testing, there may not be any difference in the comparative figures, but in many other situations there are marked

Health laboratories are located at up to one to two days travelling distance from rural populations, and there are constraints of staff and logistic support.

differences which favour conventional techniques.

The diagnosis of bacterial infections involves incubation of inoculated culture media. This is a slow process and with mycobacteria many weeks elapse before there is any evidence of bacterial growth. Nevertheless, automatic systems that continuously monitor growth of bacteria are available (e.g. Bac-Tec). During the growth of bacteria, CO_2 production is closely monitored. This is done by in-built radiometric, spectrophotometric or fluorescence sensors. Following the detection of increased CO_2 levels, it is possible to detect the growth of ordinary bacteria within 8 hours and of mycobacteria within five days. Subculture, biochemical reactions and serotyping of the isolate are currently essential for the identification of the organism.

Information about antimicrobial susceptibility can be obtained within a few hours for ordinary bacteria and within a few days for slow-growing organisms. The price of the equipment limits its use to advanced centres. However, this should not prevent rapid diagnostics from being used in developing countries for the following reasons.

The conventional tests require special equipment and well-trained personnel for their execution. Rough handling of specimens during transportation damages the pathologic constituents in the specimens. Health laboratories are located at up to one to two days travelling distance from rural populations, and there are constraints of staff and logistic support. Encouraging the use of rapid diagnostics is the only way to provide laboratory support for clinical care and public health surveillance in such countries. This has been vindicated by the introduction of rapid diagnostic techniques for viral infections.

In most of the developing countries, the national financial allocations towards laboratory services are limited. However, with international encouragement, including technology transfer, some of the countries will become self-sufficient in the production and supply of kits. With increased production, the cost is expected to come down automatically.

Regional collaboration would ensure ready availability of cheaper but good quality kits in the Region. Rapid diagnostics are also invaluable in the management of noncommunicable diseases. Laboratory personnel in six countries of the South-East Asia Region were trained in the use of locally available rapid diagnostic techniques in virology, bacteriology and clinical chemistry during 1991 and 1992[62]. Local evaluation is desirable for monitoring the efficacy of all rapid diagnostics. The techniques might have to be modified to ensure efficient working at ambient temperatures above 40 °C or below 10 °C, which prevail in developing countries[63]. Depending upon the available resources, a country may choose its own strategy for introducing rapid diagnostic technology at each level of the laboratory.

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Annex 2

Suggested Usage of Rapid/Simple Tests at Different Levels of Laboratory*

Level 1	**Peripheral laboratory**
Level 2	**Intermediate laboratory**
Level 3	**Central/State/Provincial laboratory**

Bacterial Infections	Level 1	Level 2	Level 3
Antigen Detection			
Throat swab			
Group A Streptococci	R	L	L
Cerebrospinal fluid			
Meningitis pathogens (*Pneumococcus, meningococcus, Haemophilus influenzae,* Group B Streptococci)	M+R	L	L
Mycobacterium spp.	–	R	L
Vaginal swab			
Group B Streptococci	–	R+L	L
Exudate/High vaginal swab			
Gonococci	M+R	L	L
Stool			
Diarrhoea			
Vibrio cholerae	M+R	L	L
Clostridium difficile	–	R+L	L
Salmonella spp.	–	R+L	L

M	*Microscopy*
R	*Rapid test*
L	*Conventional laboratory techniques (culture, etc.)*
*	*May be appropriately modified in view of local disease pattern and facilities available*

Bacterial Infections	Level 1	Level 2	Level 3
Blood			
Enteric fevers			
Salmonellae	R	L	L
Sputum			
Mycobacterium tuberculosis	M	L+R	L
Antibody Detection			
Typhoid	R	L	L
Syphilis (reaginic)	R	L	L
Rheumatic fever		R+L	L
Antistreptolysin-O	R		
C-reactive protein	–	R	R
Leprosy	–	R	R
Bordetella pertussis (for vaccine monitoring)	–	R	R
Tetanus (for vaccine monitoring)	–	R	R
Diptheria (for vaccine monitoring)	–	R	R
Mycotic Infections			
Antigen Detection			
Cryptococcus	M	R+L	L
Candida	M	R+L	L
Aspergillosis	M	R+L	L
Parasitic Infections			
Antigen Detection			
Amoebiasis	M	R	L
Cryptosporidium	–	R	R+L
Giardia	M	R	L
Malaria	M	R+L	R+L
Antibody Detection			
Amoebiasis	–	R	R+L
Toxoplasmosis	–	R	R+L
Leishmania	–	R	R+L
Cysticercosis	–	R	R+L
Trichinella	–	R	R+L
Echinococcosis	–	R	R

Viral Infections	Level 1	Level 2	Level 3
Antigen Detection			
Hepatitis B surface antigen	–	R	R
Rotavirus	–	R	R
Influenza virus	–	R	R+L
Respiratory syncytial virus	–	R	R+L
Herpes simplex virus	–	R	R+L
Antibody Detection			
Human immunodeficiency virus	R	R+L	R+L
Rubella	–	R	R
Cytomegalovirus	–	R	R
Varicella–zoster	–	R	R
Measles (for vaccine monitoring)	(R)	R	R+L
Dengue	R	R	R
Rabies (for vaccine monitoring)	(R)	R	R
Clinical Pathology/Haematology			
Haemoglobin	R	L	L
Sickle cell trait	–	R	R
C-reactive protein	–	R	R
Pregnancy	R	R	R
Rheumatoid factor	–	R+L	R+L
Coagulation profile	–	(R)	R+L
Tumour markers	–	–	R
CD4 cell count	–	–	R
Autoimmune disease	–	–	R+L
Sperm examination	–	M	M
Clinical Chemistry			
Urine			
Glucose	L+R	L	L
Protein	L+R	L	L
Bilirubin	R+L	L	L

Clinical Chemistry	Level 1	Level 2	Level 3
Blood			
Protein	–	L	L
Glucose	R	L	L
Bilirubin	–	L	L
Creatinine	–	L	L
Alkaline phosphatase	–	L	L
Cholesterol	–	L	L

Part III

Quality Assurance in the South-East Asia Region

Chapter 1

General Aspects of Quality Assurance

1. Introduction

QUALITY ASSURANCE (QA) in laboratory medicine is the means by which reliability, precision and accuracy of investigations used in support of optimal patient care can be achieved. Errors in analytical results may be related to various factors including the level of training of laboratory staff, quality of reagents, apparatus and specimens, and suitability of the techniques in use. Quality assurance seeks to minimize any variability in test results arising from these sources.

The issues involved in ensuring the quality of different test procedures in health care at peripheral, intermediate and central levels are discussed in this section. The role of QA in primary health care (PHC) centres is particularly critical since there are often deficiencies in the training of staff, maintenance of apparatus, and access to professional advice from larger laboratories.

Tests traditionally performed in PHC centres include conventional microscopy techniques, such as blood smears for malaria, sputum smears for tuberculosis and faecal preparations for parasites. These tests depend largely on the skill and experience of the operator, and the need for QA to control the quality of output is well recognized. Many PHC centres are now considering expanding the range of tests offered through the use of new technology in rapid/simple diagnostic tests, such as reagent strips for pregnancy, streptococcal throat infection, dengue fever, hepatitis and HIV status. Rapid tests at the PHC level may be in the form of single-use devices and some may require the use of simple meters or desktop analysers. Although such test systems are apparently simple to use, they do not automatically guarantee the quality of every test result and should be subjected to QA programmes.

2. Components of a Quality System

THERE ARE several components of a quality system. They should all be in place and operating before the end-product of good quality laboratory reports is likely to be achieved. Paying excessive attention to any of the individual components while neglecting others will not achieve lasting improvement in quality.

Quality Assurance

Quality assurance is defined as the total process whereby the quality of laboratory reports can be guaranteed. This has been summarized as

'the right result at the right time on the right specimen from the right patient, with result interpretation based on correct reference data'; to this may be added 'at the right price'.

QA comprises all the various measures taken to ensure reliability of investigations. These measures start with the selection of appropriate tests and continue with the obtaining of a satisfactory sample from the right patient, followed by accurate and precise analysis, prompt and correct recording of results with appropriate interpretation, and subsequent action based on the results.

Adequate documentation forms the basis of a QA system. This helps in standardization of methods and traceability of results on individual specimens. Regular monitoring of equipment, preventive maintenance and repair when needed are other important components of QA.

Quality assurance must not be limited to the technical procedures performed in the laboratory. All those who send pathological specimens to any clinical laboratory can contribute significantly to the reliability of the results through correct specimen collection and handling. This is particularly critical for QA of microbiological examinations, and is also important for some blood tests.

Quality assurance is a process which ensures accuracy of investigations, standardization of methods and reliability of results.

A major cause of variation is inappropriate specimen collection, delay in specimens reaching the laboratory, and/or subsequent mishandling (e.g. by evaporation or contamination of inadequately stoppered specimens in hot climates). Such variations must be minimized through careful training and adequate supervision.

The quality of a laboratory test result involves more than the sum of accuracy (closeness to the real analytical value) and precision (reproducibility). The ultimate goal of a laboratory report is the delivery of optimal health care. The most important criterion in the evaluation of a laboratory report is its contribution to this goal: the diagnosis, treatment and prevention of disease. This is called 'clinical relevance' and it can only be ensured by good communication between the clinician and laboratory personnel. Speed and the cost–benefit ratio are two other important aspects of the clinical relevance of a laboratory report.

The education and training received by the personnel is a major factor affecting the quality of laboratory performance. Not only should the technical staff be well qualified, but each laboratory should implement a programme of continuing education. This should include diverse activities such as lectures, training courses, informal staff meetings and discussions, safety briefings, reviews of laboratory procedures and reading of appropriate books and journals.

Internal Quality Control

Internal quality control (IQC) refers to the set of procedures undertaken by laboratory staff for continuous and immediate monitoring of laboratory work to decide whether the results are reliable enough to be released.

The IQC procedures have an immediate effect on the laboratory performance as they check every step in the analytical process. A good IQC programme is an essential part of good laboratory practice, and will not only improve the clinical usefulness of the results but will also help to improve the confidence of the

Qualified, educated and trained technical staff are a prerequisite for quality laboratory performance.

A good internal quality control programme ensures reliable performance of a laboratory by monitoring the analytic process and ensuring reproducibility of results.

clinician in the laboratory as well as the confidence of the technicians in their own results.

Thus, IQC regulates laboratory reproducibility (measured as imprecision), enhances the credibility of the laboratory by ensuring that sequential results are comparable and maintains continuity of patient care. Most IQC procedures employ analysis of one or more defined control materials and ascertain if the results obtained are within the limits of acceptability established previously within each laboratory.

Procedures for IQC vary considerably between laboratories and disciplines. Some disciplines (e.g. clinical chemistry) employ parallel analysis of defined control material and ascertain if the results obtained are within the limits of acceptability.

In other disciplines such as microbiology, IQC is much more complex and may involve several steps: macroscopic and microscopic evaluation of the specimen, inoculation of appropriate culture media, isolation, identification and susceptibility testing of the putative pathogen, and interpretation of the results.

External Quality Assessment

External quality assessment (EQA), in contrast to IQC, compares the performance of different testing sites. This is made possible by the analysis of an identical specimen at many laboratories, followed by the comparison of individual results with those of other sites and the 'correct' answer. The process is necessarily retrospective, and provides an assessment of performance rather than a true control for each test performed on patients' specimens.

Audit

Audit is a process of critical review of the laboratory. Internal audit is a review of laboratory processes conducted by senior laboratory staff. Such reviews are aimed at measuring various parameters of performance, such as punctuality, accuracy and costs of reports, and identifying weak points in the system.

Audit is a critical review of the functioning of the laboratory and evaluation of its services.

External audit widens the input by involving others in the evaluation of laboratory services. The users of laboratory services (usually clinical colleagues) are asked how they perceive the quality and relevance of the service provided. Comparison of methods, working practices, costs and workload between laboratories through regular discussions between heads of laboratories forms a part of external audit.

Accreditation of Laboratories

Accreditation of laboratories is a process of inspection of laboratories by a third party to ensure conformity with pre-defined criteria. Factors which may be considered are numbers and qualifications of staff, facilities available, procedures used, evidence of QA and quality control procedures, participation in EQA schemes, adequacy of documentation, reporting procedures, safety, communication within the laboratory and with users, and management structure.

Accreditation is commonly based initially on written statements of conformity (with reasons for any

Accreditation of laboratories is a process of inspection of laboratories by a third party to ensure conformity with pre-defined criteria.

nonconformity) by laboratories, followed by inspection to verify them. Accreditation may be linked to a formal system of licensing, whereby only accredited laboratories are legally entitled to practice (or to receive payment for their services), or may be a voluntary system.

Validation of Results

Validation of results is an attempt to measure quality by re-examination of specimens. This may be done by formal referral procedures, where results obtained with specimens submitted to reference laboratories are checked against the results of the sending laboratory.

Another, more expensive approach is to select fixed percentages of negative and positive specimens reported by a testing laboratory and re-examine them in a reference laboratory.

Good Laboratory Practice

IQC and EQA are concerned specifically with actual test procedures. Other parts of the process also require control if tests are to be reliable, and attention must be paid to the following aspects of laboratory practice:

- Blood collection tubes
- Phlebotomy
- Identification of specimens
- Transport of specimens
- Laboratory records
- Reports
- Contact with users

Blood collection tubes

These should be of the correct type and have the appropriate type and concentration of anticoagulant (if required).

Phlebotomy

This should be performed by standardized procedures, with the subject at rest and minimal use of a tourniquet.

Identification of specimens

All specimens and request forms should be adequately identified and hazardous specimens should be specially identified.

Transport of specimens

Transport to the laboratory should be prompt, and specimens should be kept at appropriate temperatures to minimize deterioration before analysis.

Laboratory records

The arrival of specimens in the laboratory should be recorded and specimens checked for leakage, sufficiency of volume and correctness of tube. Laboratory reference numbers, specimen identification and tests required should be properly registered; and specimens should be identified as immediate, urgent or routine.

Reports

Data processing of results, transcription to report forms and scrutiny by a senior member of staff should take place before the release of reports. The reports should be delivered to the correct location without undue delay.

Contact with users

There should be regular contact with users of the laboratory to ensure that reports arrive in time and that the results are presented in a clear and unambiguous form. There should also be discussions on test selection, taking into account the clinical relevance of tests, introduction of new tests and evaluations of the clinical benefit as well as cost.

Good Manufacturing Practice

Good manufacturing practice (GMP) is the system by which manufacturers of reagents and equipment ensure the quality of their

products. The various components of GMP include traceability of components and processes, documentation, quality control of components and products independent of the manufacturing procedures, adequate facilities, conformity with safety regulations and proper labelling, packaging and product information. GMP is relevant to clinical laboratories in two ways:

1. Where alternatives exist, reagents and equipment should be purchased from manufacturers who can demonstrate that they follow GMP.
2. Some laboratories act as manufacturers by producing reagents, control materials or quality assessment materials. Such laboratories need to apply GMP to their manufacturing activities.

Training and Education

Training and education of the laboratory worker probably represents the single most important component in a quality assurance programme. Issues to be addressed include national policies and curricula for the training of pathologists, scientists and technicians, during both primary training and in-service training. Professional status and career development are related factors. Training of medical students and nurses in the appropriate and effective use of laboratory facilities is also important.

Training and education of the laboratory worker represents an important component in a quality assurance programme.

Training needs should be continually monitored, and courses and workshops may be introduced where new needs arise (such as introduction of new methods or technologies), or where quality assessment programmes reveal the need for improvement.

Evaluation

Evaluation of appropriate reagents and equipment for use within a country may make significant contributions to the overall quality.

The choice of equipment and reagents requires considerable thought and coordination and may be cost–effective in reducing duplication of effort and preventing repetition of expensive mistakes.

It cannot be assumed that equipment and assays designed for use in one country or continent will perform well in another. Thus, for example, equipment manufactured for use in Europe or North America will be designed assuming that stable, continuous electricity is available, and may prove fragile and unreliable in the absence of such conditions.

Equipment should be selected according to rational criteria which might include cost of purchase, revenue consequences, appropriateness of the technology, robustness under local operating conditions, level of skill required to operate and availability of spares and repair/support services.

Likewise, the choice of diagnostic assays should be made after considering similar factors, such as the effect of transport time and storage conditions, including humidity and temperature, and the degree of skill required to read the assay in the PHC setting. The predictive value of tests will depend on the sensitivity and specificity under local conditions with the local population and these must be ascertained before valid interpretations can be made.

Unfortunately, initial evaluation cannot guarantee the quality of subsequent batches of reagents and some system of continual monitoring is essential.

ALL COMPONENTS of QA must be in place before the quality of laboratory results can be guaranteed. All countries need to take account of the limited availability of financial resources and select the most cost-effective measures as well as phase in their introduction.

While introducing or extending QA into any country, the primary emphasis should not be on establishing National External Quality Assessment Schemes (NEQASs) in the absence of adequate supporting QA and IQC activities. IQC and EQA are not alternatives; they serve different but complementary functions.

Undoubtedly, the introduction of EQA can stimulate improvement in quality, but prior experience indicates that EQASs alone have not been successful in producing major, sustained improvements. EQA provides a useful indicator of national standards and provides individual laboratories with assessment of their performance in terms of between-laboratory agreement. This is valuable in stimulating and monitoring subsequent improvement, but only effective QA and IQC will provide the infrastructure by which such improvements can be made.

While introducing QA, appropriate training must be provided to acquaint laboratory professionals with the basic concepts of quality programmes. Such training must be aimed at bringing about changes in management practices to ensure the optimal use of existing resources, introduction of good laboratory practice, and intelligent interpretation of data from IQC and EQA programmes.

Simple educational materials should be widely circulated. The simplicity and low cost of many of the QA techniques should be emphasized.

Internal Quality Control

IQC in laboratory medicine was developed by adapting control systems used in the manufacturing industry.

In clinical laboratory practice, quality control material must be included with each batch of patients' specimens to 'probe' the performance of an analytical system.

In clinical laboratory practice, quality control material must be included with each batch of patients' specimens to 'probe' the performance of an analytical system. Various procedures have been developed for the statistical interpretation of control data generated from quantitative (numerical) analyses, though these may not be directly transferable to establishments carrying out decentralized ('bedside', 'near-patient') testing. Some systems used in decentralized testing either do not require any calibration ('standardization') by the user as they are factory-calibrated or require infrequent recalibration.

Much of the variability of results from these methods originates from variations in operator technique. The analysis of an appropriate control material before starting and during analysis of pathological specimens can provide reassurance that the system and operator are working correctly. It is essential that the results obtained with control materials are recorded; representation in graphical form is often useful.

For quantitative analysis, control results must be compared with the acceptance limits determined from previous experience. It is common to set two limits, the first a 'warning'

limit indicating that the system is moving out of control and requires investigation, and the second an 'action' limit, indicating that the system is seriously out of control and that results from that batch should not be reported.

Written procedures must be kept for the interpretation of control data and the action required in case of failure. Learning from the causes of failure is an important part of QA as it helps to prevent recurrence.

Most IQC procedures require a QC material which must be stable and reliable. Production of appropriate QC material should be considered within a developing country rather than importing expensive material from developed countries. Simple and cheap methods, such as stabilization of human or animal serum with ethylene glycol have been described (see section on clinical chemistry).

It is unwise to rely totally on control material provided by the manufacturer of a diagnostic assay, as these are designed to control individual reagent batches and do not provide continuity of control between batches of reagent.

The use of an independent control will allow comparison between batches and provide assurance of the reliability of the product.

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All control materials are not suitable for all systems as they are essentially artificial, stabilized materials which are not necessarily compatible with the design features of all assays.

External Quality Assessment

International external quality assessment schemes

The World Health Assembly resolution WHA40.30 emphasized the importance of providing continual quality monitoring of health care by Member Countries. Accordingly, WHO has established international external quality assessment schemes (IEQAS) in several disciplines with the objective of identifying laboratories in participating countries.

While maintaining high standards of laboratory investigative capacity, these laboratories could themselves initiate QA and EQA programmes in other laboratories within their countries. The ultimate aim is to provide all the laboratories in a country with QA and EQA procedures, using networking where necessary.

The WHO International External Quality Assessment Scheme (IEQAS) operates in five sections:

1. Haematology (Watford, UK)
2. Coagulation (Sheffield, UK)
3. Clinical chemistry (Birmingham, UK)
4. Microbiology (Leuven, Belgium)
5. Parasitology (Paris, France) and immunology (Dusseldorf, Germany).

These are organized individually by the directors of the appropriate WHO International Collaborating Centres for quality assessment, and the system as a whole is coordinated by the Health Laboratory Technology Unit (LAB) at WHO headquarters with the cooperation of WHO regional offices.

An IEQAS for HIV serology was also initiated in 1989, and is coordinated by the diagnostics unit of the Global Programme on AIDS (WHO, Geneva).

National external quality assessment schemes

EQA is normally conducted through National External Quality Assessment Schemes (NEQAS) and targeted at the main investigations in the laboratory. There are also regional schemes and those run by commercial

organizations. Some schemes are provided by manufacturers for users of their equipment. These may serve to supplement independent schemes but no laboratory should be dependent on them. The basic process by which schemes operate is shown in Figure 1.

These schemes provide participating laboratories with batches of one or more assessment specimens on a regular basis. Participants examine the specimens by their routine methods and return their results to the organizing centre. The organizing centre then assesses the results and issues a report comparing individual laboratory performance with the overall national performance, often using a scoring system for performance assessment. The participating laboratory then assesses this information in conjunction with its IQC performance and other relevant data, using professional judgement to determine what action is needed. There are considerable resource implications in organizing a successful NEQAS. Organizing laboratories must be adequately funded on an ongoing basis.

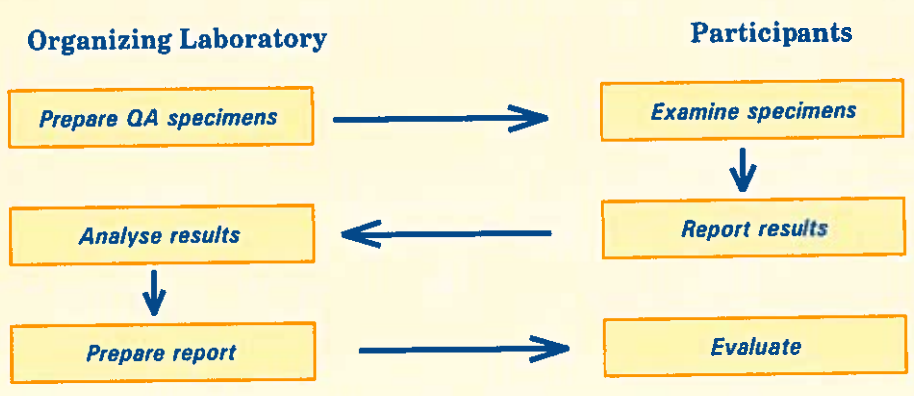
The organizing laboratory must have technical expertise, appropriate staffing, access to a good selection of sera, cultures and specimens, sufficient glassware, and necessary computing and reprographic facilities. The national government should support schemes as an ongoing programme and provide a national project with one organizing laboratory at the central level. The ultimate aim should be to cover all laboratories in the country, both in the government and private sectors. In some countries, participation in quality assessment schemes is mandatory for licensing.

Free participation encourages laboratories to participate in the programme. This is a very important issue in countries which are about to commence QA programmes, and ideally the ministries of health (especially in developing countries) should allocate funds in national budgets to finance the programmes. This investment is more than justified by the benefits derived from the subsequent improvement in disease prevention and diagnosis.

Operating costs may be recovered through subscriptions from participants once the schemes become established and valued by participants.

It is also important that the organizers of NEQAS have good scientific reputation and that their peers recognize their high professional status. EQAS schemes are only successful when participants have confidence in the scientific soundness of the scheme.

Fig. 1 The Process of External Quality Assessment



4. General Recommendations for the Organization of a Network of External Quality Assessment Schemes for All Disciplines

ONE ORGANIZING laboratory should preferably have 100–200 participants. Greater participation may become unwieldy, though with adequate data-handling facilities and full-time EQA staff much larger numbers of participants can be accommodated. Fewer participants reduce the reliability of statistical analysis of the data.

The organization of networking will differ in countries according to their size. The mechanisms suggested for networking and devolvement of EQA schemes are shown in Figs. 2, 3 and 4.

Central Organizer

A central institution, preferably governmental, participating in an international EQAS, should coordinate the NEQAS in the country. In small countries, the central laboratory itself can manage EQAS for the peripheral and intermediate levels of laboratory.

In medium-size countries a three-tier system may be necessary, while in larger countries a four-tier system may be introduced.

The country may have several regional laboratories, which could participate in IEQAS as well as manage the EQAS of intermediate laboratories in that particular region. In such countries, the national laboratory at the centre may also participate in IEQAS, but may act as an advisory body for regional laboratories.

If IEQAS participation is not available for regional laboratories of a country, the national/central laboratory may perform this function. Some intermediate level laboratories should manage EQAS programmes for peripheral laboratories.

Whatever the local arrangements in the country necessitated by the structure of health services, the principle of covering all the laboratories should be followed while ensuring that the managing (organizing) laboratories themselves participate in the IEQAS programme.

Advisory Committees

For successful implementation of EQA schemes in a country, a national advisory committee should be constituted, consisting of representatives of professional associations, university academicians, health insurance agencies, practising professionals from participating laboratories and government officials.

In addition to a national advisory committee for all laboratory disciplines, national advisory committees for each discipline may also be constituted. The central advisory committee should lay down policy guidelines for all disciplines and meet at least once a year to discuss the previous year's reports, analyse the relative performances, set priorities and initiate new programmes for the coming years.

The advisory committee for each discipline may meet at least once a year and technically review the previous year's reports. In addition, they may lay down policy guidelines, provide technical supervision and advise laboratories with persistent problems.

For successful NEQAS a laboratory should have adequate technical expertise, staffing, materials and methods for analysing results.

Fig. 2 NEQAS Networking in Small-size Countries

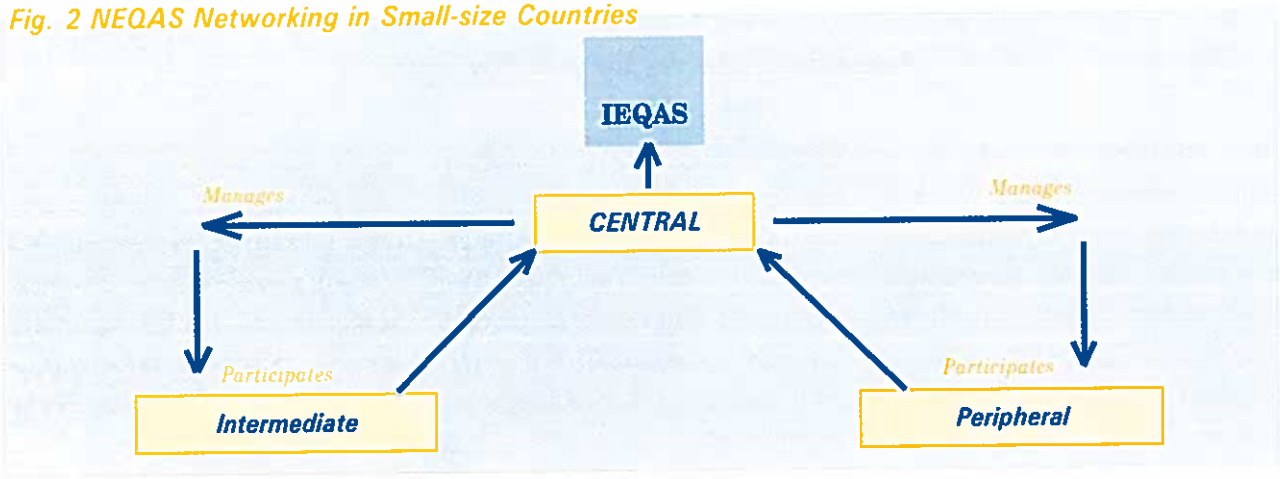


Fig. 3 NEQAS Networking in Medium-size Countries

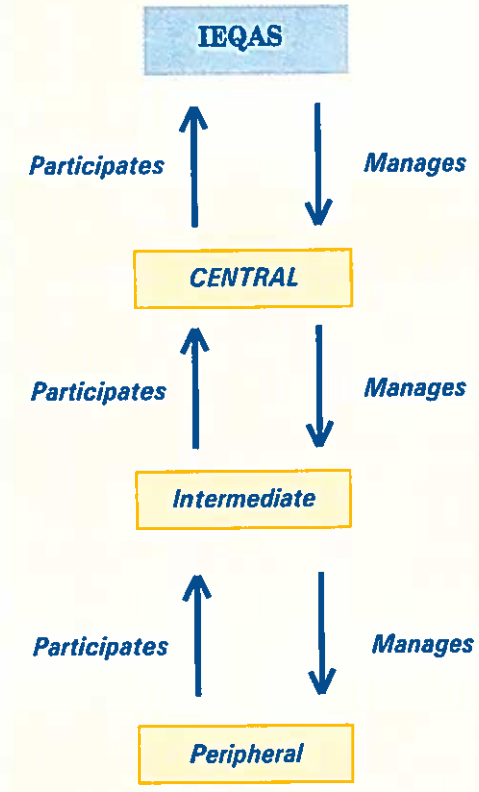
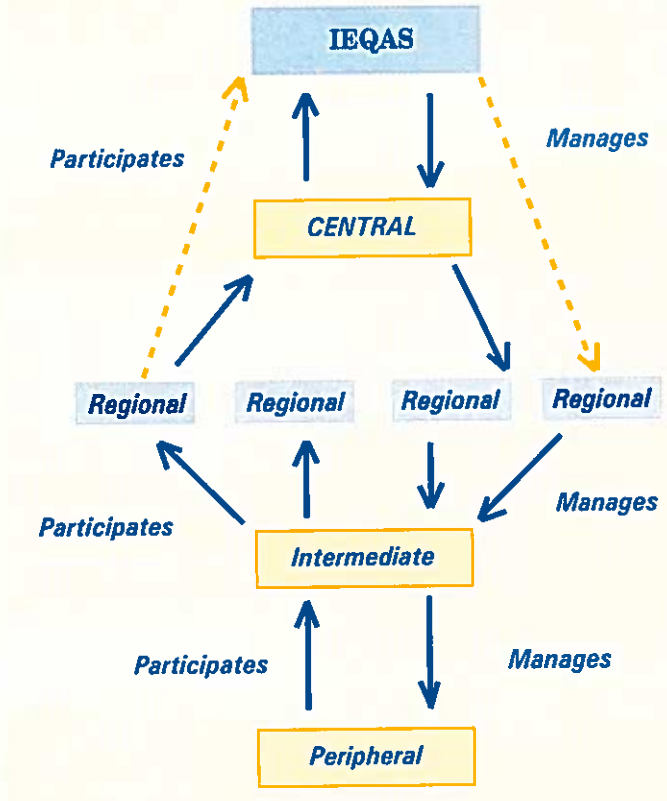


Fig. 4 NEQAS Networking in Large-size Countries



Voluntary versus Mandatory Participation

Every country must consider the local circumstances before deciding on voluntary or mandatory participation by laboratories of different levels.

There are advantages in inviting laboratories to participate in new schemes on a voluntary basis to let the participants understand the aims and value of the programme and acquire technical competence. Later, a change to mandatory participation for all, including private laboratories, can be considered. Participants must, however, be made aware of the situation.

There is a growing consensus that once a scheme is firmly established and has gained the confidence of the profession, participation should be made mandatory for all clinical laboratories that provide patient services.

This is particularly true for government-financed laboratories and for those receiving reimbursement through a social security agency.

Although private laboratories are often the least inclined to participate, they can greatly benefit from the educational opportunities offered by EQAS.

Confidentiality

The participating laboratories should be identified by a code system. Only the person or the group of persons engaged in analysing the EQA results should know the identity of the laboratory.

The identity of every laboratory, as well as details of individual laboratory performance, should be confidential, and revealed to a third party only by permission of that participant, or to a specified authority only if so agreed on registration. In addition, participants must be made aware of the conditions when they join the scheme, and before any change is considered.

5. Scope of the Schemes

QUALITY ASSESSMENT should initially address those situations where the quality of laboratory testing is most likely to affect diagnosis and treatment of diseases of major public health or clinical significance.

When initiating a quality assessment scheme, it is wise to start with specimens that are easy to prepare and do not present great technical challenges. Examples include blood slides for diagnosis of malaria and smears for examination for AAFB.

Initially, quality assessment schemes may be instituted in the fields of clinical chemistry, clinical microbiology and haematology. The number and type of analytical procedures submitted for EQA will be governed by the performance capacity of the participating laboratories. A list of suggested EQA tests for different levels of laboratories is given in Table 1.

EQA schemes and surveys should be designed to offer a realistic challenge, e.g. for microbiology testing evaluations, mixed cultures (lyophilized), which mimic the situation encountered in clinical specimens and require isolation of pathogens from commensals are ideal, rather than cultures of single organisms for identification.

Preparation of materials for EQA and IQC is described in subsequent sections.

Table 1

External quality assessment scheme analyses for different laboratories: Peripheral, Intermediate, Central

Peripheral	Intermediate	Central
Clinical chemistry		
Urine –glucose	–same as peripheral laboratory	–same
–protein	–same	–same
Blood –glucose	Blood cholesterol, urea, creatinine, uric acid, AST (SGOT), ALT (SGPT)	–same Blood–HDL, LDL, tri-glycerides, Na, K, Ca, chloride, Mg CSF–protein, glucose Urine–creatinine, steroids
Clinical microbiology		
Bacteriology		
Exudate smears	same + culture, antibiotic sensitivity	same + antibiotic assay
Sputum smears for AFB	same + culture	same + antibiotic assay
Parasitology		
Blood smears for MP (microfilariae, trypanosomes)	same	–
Faecal suspension for parasites	same + staining for *Cryptosporidium*, *Pneumocystis*	same
Bacterial immunology		
–	STS, serology for typhoid, brucellosis	same
Viral immunology/HIV		
–	HIV testing HBsAg testing	HIV testing HBsAg testing
Haematology		
Hb, TLC, DLC	same + platelet count Prothrombin time Leukaemia slides	same + partial thromboplastin, fibrinogen

Frequency of Distribution

The frequency of distribution of EQA specimens is a matter of judgement. If distributions are too few, they will not allow for a sufficiently comprehensive and regular challenge and too many will place a strain on resources.

A suggested compromise is to distribute specimens four times a year for microbiology, six times a year for haematology and six to eight times a year for clinical chemistry.

Any laboratory will require sufficient recent data before taking action on EQAS data. Frequent single-specimen distributions could be ideal as they will yield independent data, but less frequent distributions of two to four specimens may be more practical if there are logistic difficulties in distribution, and these enable recovery and similar studies.

Distributions of more than four specimens may overload participant laboratories in countries of the Region.

Treatment of EQA Specimens by Participants

The scheme can only be of help if the EQA specimens are treated as far as possible in the same way as patients' specimens. This means the specimens must be tested by the same staff who would usually test equivalent patients' specimens, using exactly the same protocols and test procedures as would normally be used.

It is common knowledge that some laboratories reserve special treatment for these specimens, using only senior staff to examine them and exchanging information with colleagues in other laboratories. This is an incorrect and perhaps irresponsible practice, as these laboratories will be falsely reassured about their apparently higher standards.

Turn-round Time

Participants should be encouraged to examine specimens as soon as possible after receipt. Unfortunately, because of the possibility of postal delays, excessively long periods may have to be allowed between dispatch of EQA specimens from the organizing laboratory and return of reports from participants.

Ideally, specimens should reach participating laboratories within two weeks of dispatch. They should be processed and results returned within two weeks of receipt. Specimens must therefore be stable enough to survive unaltered during transport to participants. An analysis of results and evaluation of performance should be sent to participants within two weeks of receipt of their results.

Transport of Specimens

Organizers usually rely on national postal services for transport of EQA specimens to participants. Care must be taken, especially with infectious EQA specimens, to see that the packing ensures the safety of postal workers, the general public and recipients of the specimens.

External quality assessment schemes will be successful only if the EQA specimens are tested by the staff who test patient samples using the same protocols and test procedures.

Some countries may have regulations concerning the transmission of pathological material by post. These should be strictly complied with, both in the interests of safety and in maintaining good relations with the postal authorities. If specimens are sent abroad by air, packing and documentation must comply with the regulations of the International Air Transport Association.

Documentation

Participants must be

- instructed on how to handle the specimens and make their reports.
- provided forms on which to record their results and return them to the organizing laboratory.
- notified of the correct or intended results for quality assessment specimens as soon as possible after the final date for return of reports to the organizing centre. This enables participants to compare their results with the intended results while the matter is still fresh in their minds, and request repeat specimens to enable them to investigate any reasons for failure.

Assessment and Improvement of Performance

For a scheme to be useful, some measure of assessment of performance must be in place. This will provide information about national levels of performance and help individual participants to evaluate their own performances and, if necessary, attempt to improve them.

The time period between the examination of EQAS specimens and the receipt of the organizer's preliminary report with the 'expected correct results' is within two weeks of the closure of the survey. The full report with supplementary documents, e.g. teaching material, is mailed later, possibly with the specimens for the next surveys if these are provided at relatively short intervals. This rapid feedback will enable the participant to repeat the examination or to identify the cause of inadequate performance.

On the other hand, a careful statistical analysis of the results and the preparation of a detailed report on the survey, including educational comments, may take considerable time.

Whatever system is used, it is important that it is clear and easily understood by participants and alerts them quickly to any deficiencies in performance.

Individual participants need two measures of their performance: one with the current distribution of QA specimens and the other over a period of time with successive distributions. Employing various statistical techniques, most systems used for presenting performance data make some form of comparison of results of individual participants with those at the national level.

Whatever system is used, it is important that it is clear and easily understood by participants and alerts them quickly to any deficiencies in performance. If a laboratory obtains poor or inaccurate results, the organizing laboratory should provide technical help by way of site visits or training of staff at the reference laboratory. The need to provide teaching material and/or organize training courses is indicated when errors are widespread and repeated among participants.

It is important to start a QA programme in a country even if only on a modest scale. NEQAS may also be started with a few participants.

As the experience of organizing EQAS grows and more and more laboratories are motivated to join the scheme, it may be enlarged into a network with central coordination, preferably with government agreement and a professional advisory committee.

7. Guidelines for Inspection of Laboratories for Accreditation

ACCREDITATION IS A procedure by which an authoritative body gives formal recognition that a laboratory is competent to carry out specific tasks. Some countries have made it mandatory for laboratories to be inspected and accredited before they can start operating.

There are various national and international standards for accreditation, some designed for general purposes, such as the ISO 9000 series and some specifically for laboratory accreditation, such as ISO 25. In several countries, specific standards for clinical laboratories have been implemented.

Elements of an Accreditation System

- An Accreditation Board/Authority which accords accreditation.
- A set of standards that must be adhered to by the applicant laboratories.
- Inspectors/Assessors appointed by the Accreditation Board.

Sequence of events

- Application by the laboratory.
- List of standards supplied by the Authority for the laboratory to declare compliance with.
- Laboratory submits data on compliance with required standards.
- Authority assigns Inspecting Team.
- Inspectors assess the laboratory and send a report to the Authority.
- Authority reviews the report and grants Accreditation or asks for corrective actions, if any, before granting Accreditation.
- Reassessment every 2–3 years.
- Inspectors may be appointed from practising medical, clinical and biomedical scientists who have adequate laboratory experience and have been trained in audit techniques.

Standards used by Clinical Pathology Accreditation (UK)

There are 44 standards drawn up under the following heads:

A. Organization and Administration
B. Staffing and Direction
C. Facilities and Equipment
D. Policies and Procedures
E. Staff Development and Education
F. Evaluation

The Inspector may assess the laboratory for compliance of the standards laid down. Inspection is not only a means to ensure compliance but is seen as an educational process in which the Inspector explains the standards and makes suggestions for correction of various deficiencies. Inspectors report to the regulatory authority regarding compliance of the standards noted above and recommend accreditation or otherwise of the laboratory.

Standards for inspection of laboratories

A. Organization and Administration

1. The Laboratory should have a document describing its organization, scope of work and strategy.
2. There is a documented line of managerial accountability from the head of the laboratory to senior management of the organization.
3. There are formal arrangements for meetings between senior laboratory staff and management to review the service, set objectives and make appropriate financial arrangements.

B. Staffing and Direction

4. Each discipline is professionally directed by a consultant pathologist or clinical scientist of equivalent status.
5. There are appropriate numbers of staff with the required training to ensure a satisfactory operation of service.
6. There is a documented line of accountability for all staff.
7. The duties and responsibilities of all staff are specified in job descriptions, and they should understand their responsibilities.
8. All staff members have a contract of employment which clearly states terms and conditions of service. Records of service of every staff member are maintained.
9. Regular staff meetings are held and minuted to review services.
10. All new staff members are given a comprehensive orientation and induction programme including health and safety.

C. Facilities and Equipment

11. There is adequate office and laboratory space.
12. Where applicable, there are adequate mortuary and postmortem facilities.
13. Staff facilities are suitably located.
14. There are adequate facilities for patients, such as waiting area, phlebotomy room, separate toilets.
15. There is appropriate space available for specimen reception, dispatch and handling.
16. There are appropriate and adequate data storage, retrieval and communication facilities. A confidential, fire-proof computer system should be available.
17. The laboratory equipment meets the demands of the service and is properly maintained. Log books

and maintenance standard operating procedures (SOPs) are available.

18. There is adequate and safe provision of lighting, heating, ventilation, power, gases, water and drainage. SOPs for disposal are maintained.
19. There are adequate storage facilities for specimens, reagents and records.
20. There are adequate and secure storage facilities for blood and blood products.
21. The working environment is safe and in accordance with current legislation.

D. Policies and Procedures

22. There is an up-to-date user manual. SOP of each technique should be available.
23. Request forms for laboratory investigations and specimen labels include provision for unique patient identification and adequate supporting information.
24. Reports of laboratory results are validated prior to dispatch, are timely, and include unique patient identity, date of testing and name of requesting clinician.
25. Interpretive reports are accurate, comprehensive and clinically relevant.
26. There are written procedures relating to specimen collection, handling, retention and disposal.
27. If the hospital where the department is sited is a potential receiving centre for a major accident, there is a readily accessible document within the department instructing staff on procedure.
28. There are records of all reagents, calibration and quality control of materials.
29. There is a written, signed and dated procedure for performance of each test.
30. There is a written procedure for reporting of each test.
31. There should be a written procedure for oral transmission of results. In view of advanced communication facilities, phones, faxes, e-mails may be used.
32. There is a written procedure for regular maintenance of equipment.
33. There are written procedures for decontamination of all items of equipment and working space.
34. There is a written policy describing any out-of-hours service.
35. In hospitals, a nominated consultant in the microbiology department is responsible for infection control.

E. Staff Development and Education

36. There is a written programme of training for all trainee members of staff.
37. There are appropriately sited facilities available to support training and continuing education.
38. There are resources for staff to attend appropriate seminars, meetings and conferences.
39. There is a continuing education programme for all staff.
40. There is a staff appraisal system.

F. Evaluation

41. The department must have a formal policy for IQC and must participate in recognized EQA programmes.
42. Quality assessment programmes are widely publicized in the department with regular formal review of performance.
43. QA evaluation includes continuing audit of the service provided.
44. Senior pathology staff participate in audit activities with other clinical specialties.

INSPECTION PROCESS

- Application by the laboratory.
- List of standards supplied by the Authority for the laboratory to declare compliance with.
- Laboratory submits data on compliance with required standards.
- Authority assigns Inspecting Team.
- Inspectors assess laboratory and send report to the authority.
- Authority reviews report and grants accreditation or asks for corrective actions, if any, before granting accreditation.
- Re-assessment every 2–3 years.
- Inspectors may be appointed from practising physicians, clinical scientists, biochemical scientists who have adequate experience.
- Inspections should be performed by staff trained in audit technique.
  It is best for two inspectors to work as a team, especially initially.
- The Inspector should liaise with the Quality Manager of the laboratory to be inspected when necessary.

Purpose of inspection

- The management's objectives, as defined in the quality system, are achieved.
- All members of staff perform their duties to a satisfactory level and all procedures detailed in the quality system are followed.
- The documented procedures and work instructions are followed.
- The documented system meets the requirements of the specified standards.
- The appropriate records are taken and properly filed.
- Any deficiencies (non-complaince) in the quality system, processes, practices, products or test results are identified and appropriate corrective action taken.

Inspection documentation with the Authority should include:

- Standard Operating Procedure (SOP) describing the entire inspection process.
- SOP describing training of inspectors.
- Schedule.

It is important to note that it is the quality system that is being inspected, not the staff. Any deficiency is in the quality system and not in the personnel.

- Inspection checklists.
- Inspection reports.
- Non-compliance notes.
- Summary reports for Management Review Meetings.
- Minutes of Management Review Meetings.
- Records of follow up actions from Management Review Meetings.
- Log of Inspections and of Inspectors.

Selection of inspectors

- Inspectors should be competent and meet agreed criteria, e.g. attended a training course and shown ability in performing inspections with a trained inspector. They should volunteer for the role.
- All grades of staff should inspect, although they may not all be able to inspect all aspects of the quality system. For example, a Laboratory Secretary could inspect documentation control but not undertake a vertical inspection, which requires more specialized knowledge.

Monitoring of inspectors

- Some inspectors may be too enthusiastic and somewhat insensitive.
- They should be sensitive to any complaints by inspected laboratories.
- If inspected laboratories are concerned about an Inspector, they should initially talk to them to point out their concern. This would probably be sufficient to make them change their approach.
- If after this, they still do not show the right approach, then it is probably best for the Authority to dispense with their services.

Requirements of inspection

Inspections must be:

- **Scheduled**—inspectors should plan in advance and laboratories should know when they are to be inspected.
- **Structured**—according to agreed procedures.
- **Preplanned**—use a pre-prepared checklist.
- **Independent**—inspectors should not inspect an area they work in.
- **Objective**—recording only substantiated objective evidence.
- **Competent**—conducted by trained inspectors who act professionally.

Structure of inspections

- Inspections must follow a similar pattern.
- This will benefit the Inspector and the inspected laboratory will know what to expect.

Independence of inspectors

- Inspectors should be independent of the area they are inspecting.
- This should not cause a problem in a large institute although it can lead to certain problems.
- The inspector must act professionally at all times, building trust through tact and confidentiality.

Objectivity

- The inspector must always objectively record the substantiated objective evidence.
- If they see a potential problem, they should ask the appropriate person about it, at that time or later, to verify their observations.

Competence

Inspections should be conducted by trained inspectors who act professionally.

Acting professionally means showing

- —Tact
- —Thoroughness
- —Persistence
- —Technical competence
- —Good questioning/interviewing skills
- —Objectivity
- —Fairness
- —Confidentiality.

Performing the inspection

- Use the pre-prepared checklist and the filled proforma by the laboratory to be inspected as a guide when performing the inspection.
- Record details of observations made as the inspection is being performed. Do not rely on memory.
- Information may be gathered by watching staff at work, interviewing them, inspecting the work place, e.g. contents of refrigerators, freezers, etc. and by examining documentation, both SOPs and work records.

  During the inspection, the inspectors may wish to meet to discuss their findings.

  There may be possible problems they wish to talk about or information which they feel will help the other inspector as they continue their inspection.

After the inspection

- Following the inspection the inspectors should meet to discuss observations and prepare non-compliance notes (NCNs). These are used to report any deficiencies found during an inspection.

- The inspector should have the final say if there is any disagreement about the interpretation of an observation.
- The advice of the quality manager of the inspected laboratory may be sought at any time.

Writing the report

- The report need only be a brief statement summarizing the findings.
- The important details should be given in noncompliance reports.
- All positive findings should be highlighted.
- The report should also contain any suggestions for quality improvements.

Noncompliance notes

Noncompliance notes (NCNs) are used to report any deficiencies found during an inspection.

The purpose of the NCN is to

- Convey to the inspected laboratory the findings in a clear and accurate manner so that they know precisely what was wrong and what to do next.
- To advise the next inspector what you have found so that they can follow it up.
- To present a record that can be reassessed at a later date during management review.

Examples of noncompliance

Documentation

- No record that SOP was reviewed on schedule.
- An unauthorized photocopy of an unauthorized SOP found.
- An authorized SOP has hand-written changes.
- A new version of an SOP has been authorized but the control of change procedure has not been followed (not Control of Change form).
- Following an old version of an SOP.
- Conducting a routine test for which an SOP is not available.

Staff training

- A relatively new member of staff does not have a training book.
- A relatively new member of staff does not know the name of the Laboratory Safety Officer.
- A relatively new member of staff does not know the fire-drill, i.e. where to go in the event of a fire bell or which route to take to get there.
- There is no evidence that a section has regular local staff meetings.

Inspection documentation

Examination of documentation is an important part of all inspections. There is no set procedure for examining documentation.

Checking equipment status

This should be easy to do. All equipment should have an inventory number; it should have a label saying it has passed an electrical safety test; if appropriate, it should have a monitoring form attached or calibration status detailed.

Observing processes

- If possible, take a 'fly on the wall' approach.
- Try not to make it obvious that you are watching someone handle a sample. They may become so nervous that they make an error or they will make extra effort to do it right.
- Inspectors report to the regulatory authority about the compliance of the standards and recommend accreditation or otherwise of the laboratory, with recommendations for corrective action (if deemed necessary) with the time-frame.

Maintaining accredited status

Re-inspection every two or three years as external audit with the same inspection process.

National Steering Comittee for Quality Assurance in Laboratory Medicine

Membership

- Experts in diverse disciplines of laboratory medicine
- Representatives of professional bodies
- Clinicians
- Representatives of the government.

Duties/Responsibilities

- Set up programmes in different disciplines
- Select members of Advisory Committees of each discipline
- Liaise with the Advisory Committee of each discipline
- Annually review the QA programme in the country
- Give advice on educational programmes
- Liaise with the National Steering Committee.

National Advisory Committees for each Discipline of Laboratory Medicine

Membership

- Experts in diverse disciplines of laboratory medicine
- Representatives of professional bodies
- Representatives of the government

Duties/Responsibilities

- Select organizers of the EQA programme
- Set up programme
- Set up networking
- Review results annually
- Give advice to poor performers
- Prepare educational programmes
- Prepare SOPs for laboratory techniques.

Management and Control Process of Accreditation

National Authority for Accreditation of Laboratories

Under government control

Membership

- Representative of the government
- Experts from diverse disciplines
- Representatives of professional bodies.

Duties/Responsibilities

- Select Inspectors
- Set up programme
- Review accreditation every three months
- Liaise with the National Steering Committee regarding improving non-compliers
- Prepare national standards
- Prepare SOPs of the Inspection process, standards and education programmes.

Further Reading

Burnett David, 1996. *Understanding accreditation in Laboratory Medicine.* ACB Venture Publications Association of Clinical Biochemists, Royal College of Pathologists, 2, Carlton House Terrace, London SW1Y 5AF.

CPA Accreditation Handbook. Clinical Pathology Accreditation (UK) Ltd., 45, Rutland Park Sheffield S10 2PB.

Chapter 2

Quality Assurance in Clinical Chemistry

1. Introduction

THE PRIMARY FUNCTION of any department of clinical chemistry is to provide the clinician with analytical data obtained from examination of specimens taken from patients and to assist in interpreting these results. This helps in diagnosis and control of therapy for patients and serves public health purposes.

Quality assurance (QA) is one means of ensuring that the results issued from such a laboratory are dependable and sufficiently accurate and precise to allow decisions to be taken with confidence. If such confidence is lacking, additional costs will accrue, not only in terms of inappropriate resource utilization through repeat testing, etc. but also in terms of suffering of individual patients and, in some cases, the entire community. Quality assurance improves test reliability by minimizing the inherent variability arising from biological or analytical sources.

Quality assurance (QA) ensures that results issued from a laboratory are dependable, accurate and precise.

Overall, QA seeks to guarantee that the right result, at the right time, on the right specimen from the right patient, is interpreted using the right reference data at the right cost.

An effective QA programme, which includes appropriate QA measures and schemes for internal quality control (IQC) and external quality assessment (EQA), is therefore essential in clinical chemistry in relation to preventive, diagnostic, curative and rehabilitative medicine. To facilitate optimal patient care and avoid the adverse consequences of quality failure, QA, IQC and EQA are required in all laboratories in both developed and developing countries.

These elements of a QA programme are considered together with the selection and preparation of suitable QC materials required to carry out IQC and EQA functions.

2. Quality Assurance

QUALITY ASSURANCE, which is often equated with 'good laboratory practice' encompasses all measures taken to ensure reliability of investigations. It starts from test selection, through obtaining a satisfactory sample from the right patient, analysing it and recording the result promptly and correctly, to appropriate interpretation of and action on the result, with all procedures being documented for reference.

General QA measures are essential for providing a secure basis within which analytical quality control can be effective.

In addition to the aspects mentioned above, any testing site must employ well-chosen, reliable equipment and methods, which should be carried out by trained, competent and motivated staff on correctly-collected specimens, in an environment which is safe, clean, well-lit and appropriate to the task, and provide results which are recorded correctly.

Test Selection and Siting

In any situation where investigations are required, it is essential to critically examine the circumstances and requirements. The clinician and laboratory should then come to an agreement as to which investigations should be carried out locally and which by a more distant laboratory. This would ensure that all investigations are relevant and reliable, with appropriate resourcing to assure quality.

An increasing number of laboratory tests (mainly quantitative biochemical tests) are now being considered for use in peripheral sites, and employ 'desktop' analysers or single-use devices designed for use in primary care. Although these are apparently simple to use, the quality of results is not ensured.

An intermediate or central level laboratory should provide continuous support and follow-up for peripheral laboratories to resolve difficulties rapidly and effectively.

It is the responsibility of an intermediate or central laboratory to act as a mentor for peripheral laboratories by providing continuous support and follow-up to resolve difficulties rapidly and effectively.

Such support should encompass assistance with system selection (standardization of products used can be helpful), site assessment, operator training, equipment maintenance and an effective analytical QA programme including IQC and EQA. This support should allow for consultation and personal visits to the testing site for consultation, calibration and maintenance.

Support in this manner must be costed realistically. Though such networked testing systems with appropriate QA mechanisms appear to require more resources than analysis in a central laboratory, they minimize the need for patient or specimen transport.

Nevertheless, quality is essential wherever measurements are made; and though quality may be expensive, the cost of poor quality is greater through both resource usage and personal suffering.

Many organizations have made recommendations regarding the repertoire of tests required at each laboratory level. Such recommendations must be interpreted with due consideration of local circumstances (finance, facilities available, predominant public health problems, communication). The following may provide a useful guide:

Peripheral level

Qualitative tests for urine (glucose, albumin) and blood sugar.

Intermediate level

Tests for bilirubin, glucose, urea, creatinine, uric acid, AST (SGOT), ALT (SGPT), cholesterol.

Central level

Blood/serum tests as mentioned above, plus estimation of HDL, LDL, triglycerides, Na, K, Ca, chloride, Mg, alkaline phosphatase.

Specimen Collection and Transport

The quality of the specimen dictates the quality of the results obtained; it is not possible to obtain useful

results on an incorrectly-collected or deteriorated specimen, however good the analytical quality (though it is of course possible to get poor results on a reliable specimen). The laboratory is responsible for ensuring that the specimens are collected by staff who are well trained and reliable, and that specimens do not deteriorate during transportation.

Laboratory Facilities and Management

Laboratory facilities should be adequate for the work carried out. Sufficient equipment and glassware should be available. It should be kept clean and maintained well, with the equipment in good working order. To permit full and appropriate use of all equipment, sufficient spare parts and expertise in maintenance must be available. The exact requirements depend on the level of laboratory and the test repertoire.

Laboratory management must be good. This means that all resources (personnel, equipment, facilities, finance and raw materials such as blood) are used appropriately and effectively for the patients' benefit. Analytical procedures used must be reliable and documented fully. Quality control measures and managerial procedures should also be documented.

Staff Selection, Training and Motivation

A laboratory's greatest asset (or liability) is its staff. Staff should be selected on the basis of their aptitude, motivation and potential, given appropriate training (including retraining when required), and motivated continually. Only in this way will the clinical chemistry laboratory be able to provide a valuable and reliable service and contribute to clinical interpretation of the investigations carried out.

Specimen and Report Identification and Plausibility

Reliable identification of specimens and reports, at all stages between the patient and the clinician, is essential for providing good quality service. The effects of transposition or transcription errors on patients can often be much greater (and potentially fatal) than the effect of analytical failures in accuracy or precision. Particular attention must be paid to the unequivocal identification (usually by labelling, since positioning may not be fully reliable) of all intermediate containers, e.g. the tube to contain serum when separated from clotted blood, the reaction tube in an analysis, in addition to the original specimen container.

Reliable identification of specimens and reports, at all stages between the patient and the clinician, is essential for providing good quality service.

Care must be exercised during the stages of recording all observations, calculating and transcribing reports to ensure that the data are correct and attributed correctly. In the calculation stages, it is helpful to assess whether the result obtained is plausible, and when reporting to compare the investigation result with the clinical details on the request form and the results of any other investigations on that patient.

Summary

Quality assurance measures mostly seek to apply sound scientific and management principles to laboratory operation. The philosophy of quality culture needs to be inculcated at all levels for achieving reliable results for quality care to the patient.

3 Internal Quality Control

INTERNAL QUALITY CONTROL assesses, in real time, whether the performance of an individual laboratory or testing site is sufficiently similar to its own previous performance for results to be used.

IQC controls reproducibility (or precision) and facilitates continuity of patient care over time. Most IQC procedures employ analysis of control materials and compare the results with preset limits of acceptability. Unsatisfactory sets of results may thereby be suppressed. Many systems used in peripheral level testing are factory-calibrated (or require infrequent recalibration), and much of the variability of results originates from variations in operator technique.

Before starting analysis of a set of specimens, the analysis of appropriate control material can provide reassurance that the system and operator are working correctly. (It must be noted that not all materials are suitable for all systems, and may produce differing results even if they are suitable, as they are essentially artificial, stabilized materials.)

It is essential, however, that the results obtained are recorded, compared with acceptance limits and that appropriate action is taken. If the results are outside the limits, then the situation requires investigation and patients' specimens should not be analysed. The necessary procedures must be documented.

Graphical presentations are helpful. Specimen collection can be a major source of variation but it is not possible to use IQC measures to control this. Variation must be minimized through careful training and supervision procedures.

Simple Procedures

Some IQC procedures are simple and inexpensive to implement. These include:

- recording and monitoring instrument readings (e.g. absorbance) using calibration materials, as a check on chemical and instrumental drift.
- analysis of one or more specimens (at different concentrations) from the previous analytical batch, as a check on assay stability (provided that the specimens are stable for that particular period).

These procedures do not require sophisticated statistical techniques or expensive materials. Any laboratory should be capable of introducing these simple steps, which can provide invaluable information on assay performance.

Statistical Quality Control Techniques

Most IQC procedures rely on introducing control specimens into each batch of analyses. These specimens must be stable and of reproducible composition, and the results obtained with them should reflect the assay's performance with patients' specimens. Graphical and statistical analyses can be applied to the results to confirm whether or not the analytical process is 'in control' and thus whether or not the patients' results can be reported.

Classically, two specimens, one with normal and one with abnormal analyte concentrations, are analysed in each batch. The results are recorded, and compared with acceptance limits. These limits are derived from initial analysis of the control specimen in 20 batches, leading to calculation of the mean ('assigned' or expected value) and the standard deviation (SD). Subsequent results may conveniently be plotted on a control chart, with concentration on the y axis and batch number (usually days) on the x axis.

This approach facilitates decision-making if horizontal lines are drawn at the mean (expected) value and at 2 SD and 3 SD above and below the mean value.

If the analysis is in control, results will be scattered randomly above and below the expected value, the distribution being such that only 1 in 20 (5%) will be more than 2 SD from the mean and only 1 in 100 (1%) more than 3 SD from the mean. Loss of precision, however, will yield a wider scatter of results, whereas loss of accuracy will cause a shift to higher or lower values. These changes can be seen on the graphical presentation ('control chart') and corrective action taken. The rules are:

Result >2 SD from mean

Warning—investigate method to avoid future problems.

Result >3 SD from mean

Action—reject batch and investigate problem before repeating analyses.

Possible causes of loss of precision

- problems with the pipetting equipment,
- variable reaction timing,
- variable reaction temperature, and
- instrument (e.g. photometer) instability.

Possible causes of loss of accuracy

- incorrect sample or reagent volume pipetted,
- incorrect reaction, timing or temperature,
- incorrect instrument setting (e.g. wavelength),
- calculation errors, and
- deterioration of calibration material.

The corrective action to be taken depends upon the type of problem, i.e. whether the method has become imprecise or inaccurate.

Validated Control Techniques

More complex and effective control rules have been formulated, notably by Westgard and co-workers, who have validated their power (to reject unsatisfactory batches and accept satisfactory batches) using computer simulation studies.

The so-called 'Westgard rules'[1] for the interpretation of control data, using two materials analysed once in each batch, have been published as a proposed selected method and are given below. If both control results

Westgard Rules

[1] *2S Warning*	One result more than 2SD from target.
[1] *3S Action*	One result more than 3SD from target.
[2] *2S Action*	Two consecutive results more than 2SD from target in same direction.
[R] *4S Action*	Difference between the two control results exceeds 4SD.
[4] *1S Action*	Four consecutive results more than 1SD from target in same
[10] *x Action*	Ten consecutive results same side of mean.

Wheeler Rules

[1] *2S Warning*	One result more than 2SD from target.
[1] *3S Action*	One result more than 3SD from target.
[2/3] *2S Action*	Two of the last three results more than 3SD from target in same direction.
[4/5] *1S Action*	Four of the last five results more than 1SD from target in same direction.
[8] *x Action*	Eight consecutive results same side of mean.

are within 2 SD from their target, the batch is accepted. If at least one control result is more than 2 SD from the target, the remaining rules are evaluated in turn, and the batch rejected if any one rule is satisfied. If none is satisfied, the batch is accepted, but the situation should be investigated before the next batch is analysed. Where only one control material is analysed in each batch, a modification known as 'Wheeler rules'[2] applies.

Techniques using Patients' Data

For laboratories with a heavier workload and access to calculation facilities, control techniques using patients' results can be helpful. These do not use QC materials, and are thus cheaper and not susceptible to errors due to matrix effects.

The mean of all patients' results for the day is calculated and used as a control parameter. Results which are very high or low, e.g. urea levels from patients with renal failure, must be excluded (though using exclusion limits which are too narrow makes the technique insensitive).

Corrections may also be needed for the proportion of ambulant and hospitalized patients for protein-bound analytes such as calcium. Though the technique is helpful, it should not be used as the sole form of IQC.

Summary

Simple but effective IQC procedures are available, and should be used universally. Without effective IQC programmes, laboratories cannot be sure that the results they produce are reliable. They may thereby hinder rather than assist reliable health care.

4. External Quality Assessment

EXTERNAL QUALITY assessment (EQA) addresses differences between testing sites, so that continuity of care is maintained geographically. Common mechanisms include the analysis of identical specimens at many laboratories, and the comparison of results with those of other sites and a 'correct' answer. The process is necessarily retrospective.

Though in most situations EQA is considered to deal primarily with the assessment of individual laboratories, it in fact provides assessment of:

- The overall standard of performance (state of the art).
- The influence of analytical procedures (methods, reagents, instruments, calibration).
- Individual laboratory performance.
- The specimens distributed in the EQA scheme.

This information can be invaluable. For example, the general standard of performance can emphasize the need for improvements in quality within a country, and the progress in improving between-laboratory agreement can be monitored. EQA data can identify methods and techniques which yield better (or worse) performance than others in routine use, and thus encourage the adoption of more reliable procedures.

The data obtained for each specimen should be compared with

previous experience in the scheme, as this can provide a valuable appraisal, in reasonable time, of the specimen distributed. If problems are apparent, then individual laboratory performances should not be assessed using this material.

Overall, EQA schemes (EQASs) should not be used for policing or licensing laboratories, but should provide independent, objective data and act as an educational stimulus for improvement. Participation in EQASs should ideally be voluntary, until confidence in the scheme's reliability has been established. Participation should also be seen as an integral part of professional standards in laboratory medicine.

Practical Considerations

In the early 1980s, the WHO Regional Office for Europe[3] addressed the practical factors associated with establishing EQASs in a publication which also identified recommendations for EQAS design and operation. These recommendations were later abstracted for developing countries by the WHO Health Laboratory Technology Division[4].

More recently, ISO/IEC Guide 43-1(Proficiency testing by inter-laboratory comparisons, Second edition, 1997) was prepared by the International Organization for Standardization, to provide guidance for EQA for any physical or chemical measurement. This provides the basis of the standards drawn up by Clinical Pathology Accreditation of EQAs in the United Kingdom.

Overall, EQA schemes should not be used for policing or licensing laboratories, but should provide independent, objective data.

The Committee on Analytical Quality of the International Federation of Clinical Chemistry's (IFCC's) education and management division has also drafted guidelines for improving analytical quality in developing countries through establishing and managing EQASs (Fundamentals for external quality assessment, IFCC).

This section contains much useful information on establishing EQASs, and the reader is referred to its latest version. Discussion includes emphasis on:

- The scheme organizer and required support facilities.
- The scope of the scheme.
- The plan for distribution frequency and turn-round times.
- Specimen distribution logistics.
- Specimen procurement.
- Instructions to participants.
- Data analysis design and implementation.
- Reporting arrangements.
- Target values and acceptability limits.
- The educational component in EQA.

Scheme Design

For an EQAS to be successful in stimulating improvement, participants must have confidence in the scientific validity of the scheme design and reliability of its operation, or they will not take action on receipt of information from the scheme.

Experience with many schemes has indicated several essential design criteria:

Sufficiency of recent data

This can be achieved through:

- Frequent distribution.
- Rapid feedback of initial performance information after analysis.

Effective communication of performance data

This can be achieved through:

- Structured, informative and intelligible reports.
- A cumulative scoring system.

An appropriate basis for assessment

This should include:

- Stable, homogeneous specimens

which behave like clinical specimens.
- Reliable and valid target values.

Target Values in External Quality Assessment

The targets (designated values) against which performance is assessed are critical. This will ensure participants' confidence in taking action based on EQA data.

The primary purpose of EQA is to eliminate bias in IQC. Similar considerations apply to value assignment for IQC materials and to designate values in EQA. Ideally, all designated values should be fully validated, but this can present major difficulties. In practice, their selection is based on a combination of prejudice, theory and practical considerations appropriate to the circumstances.

There are three main alternative approaches.

1. Definitive or reference method value.
2. Reference laboratory value.
3. Consensus value.

Definitive or reference method value (from analysis in one or more laboratories)

Definitive or reference methods give a single target value, but the other two approaches can yield overall or method-dependent values.

Definitive or reference method values are theoretically the best option, but such methods are not available for all analytes, and the cost of obtaining them may be prohibitive. To increase confidence in the resulting value, use of more than one laboratory is also desirable. However, the QC materials used in EQA and IQC often do not behave exactly as clinical specimens, and give different relationships between the values obtained by various methods. These matrix effects can thus lead to incorrect conclusions being drawn about method accuracy.

Reference laboratory value (from analysis in one or more laboratories)

The reference laboratory approach is only viable if the performance of the laboratories used is greatly superior to that of most participants, and in many cases this is not so or cannot be demonstrated.

The dangers inherent in this approach include introducing errors due to the laboratories' own bias and giving these laboratories excessive confidence in their performance. Confidence in the mean is increased by use of several laboratories' values, with rejection of outliers.

The primary purpose of EQA is to eliminate bias and improve the performance of IQC.

Consensus value (mean or median derived from participants' results)

There is no scientific reason for consensus values to be accurate, but practical experience shows that in most cases the mean of many participants' results is not only convenient (being essentially free, and available when required) but also sufficiently reliable as a target. Their validity must not, however, be assumed, but be demonstrated through repeatability, recovery and comparison studies with other EQASs wherever possible.

A pragmatic approach which is recommended is to initially use overall consensus values within a country, but obtain external validation through comparison of these values with values obtained (by consensus or reference laboratories) in developed countries to establish their credibility.

If the spread of results is wide, method-dependent targets are not justified (except for enzyme activity assays, where the result is completely dependent upon the analytical procedure), and in any case, should not be encouraged unless there is evidence of matrix effects.

Data Handling and Presentation in External Quality Assessment

Reliable and appropriate data handling is essential for any EQAS. This includes both practical and theoretical aspects which require consideration.

Data processing must be rapid and accurate for schemes to be able to provide helpful reports to participants soon after the deadline for receipt of results. In many cases, this will require a computerized system. When selecting or developing a system, several factors need to be considered, because the scheme will be totally reliant on the system.

Data reduction should be appropriate for the scheme. If consensus values are used, the relative merits of means and medians should be assessed, and outlier rejection procedures should be robust.

Scoring systems assist performance assessment. These improve the intelligibility of data by compressing results and targets for a number of specimens and/or analytes into a single figure (or set of figures), thereby enabling a cumulative assessment.

The system chosen should be robust, clinically relevant, and not affected by the performance of other participants, e.g. the Variance Index (VI) system. A well-chosen system will allow comparisons over time and geo-graphical location, both for individual laboratories and all participants. Details on the intentions of scoring, with practical examples are given in 'Scoring systems in EQA for Clinical Chemistry'.

Reports should be structured, with the most important and basic information in a summary page at the beginning. This will be of most use to laboratory directors, and can be followed by more detailed information of greater interest to the individual analysts. This structure is also helpful in other ways: if there are no problems, the participant may need only to look at the summary page, but if this indicates difficulties then the more detailed information can be examined.

It is important to recognize that scoring systems are a way of simplifying data presentation, and if there are problems then the original results must be examined. A suitable format is a graph of laboratory results (y) against target values (x).

A basic principle is that participants must be informed about the data processing and presentation used, and assistance in interpretation given on request. It is particularly important that participants are aware of how decisions based on scheme data (e.g. for laboratory accreditation or identification of 'poor performers') are made.

Summary

Effective EQASs can assess the general standard of performance in a country, stimulate recognition of the need for improvement, and monitor progress towards better between-laboratory agreement. At the same time, they provide an excellent management tool for individual laboratories, provide an independent and impartial assessment of their performance, stimulate improvement and monitor progress. If these objectives are to be successful, schemes must be properly designed and operated.

5. Materials for Internal Quality Control and External Quality Assessment

AS OUTLINED EARLIER, QC materials are required for most IQC and EQA procedures. Such materials must be fit for the intended purpose, and the main needs are:

- Homogeneity.
- Stability.
- Behaviour similar to clinical specimens.
- Availability in quantity.
- Low cost.

Clinical chemistry utilizes many blood, serum or plasma specimens. Serum is the preferred basis for QC material, in view of its greater stability.

For both IQC and EQA of most investigations, animal (e.g. bovine) serum is the best source in many countries and its use is generally recommended. Special problems arise where the analyte is species-specific (e.g. immunoglobulins) or the serum matrix is important in the separation stages (e.g. immunoassays).

Collection of Base Serum

Animal serum can be collected under aseptic conditions from special herds of cattle by venepuncture, but this will add considerably to the cost.

Equine serum has also been found to be useful and could be used, depending on the ease of availability. A competent member of staff should undertake/supervise every stage of the process, from the collection of blood to the final labelling of the finished preparation.

Alternatively, blood may be collected at an abattoir. Blood is collected from the animals (preferably steers) at the time of slaughter, by severing the great vessels of the neck so that the escaping blood can be collected into clean plastic buckets.

Only the first rush of blood should be collected and any contamination with saliva or gastric contents must be avoided. A small sample of clotted blood is set aside for assaying the brucellosis titre, and any positive reacting sera (1:80 dilution in direct agglutination test) should be discarded.

The blood is allowed to coagulate under active agitation until clotting commences. Blood is then extruded manually from the clots and the fibrin removed and discarded. This frees most of the cells and serum, thereby allowing quick separation by centrifugation. Only that amount of agitation necessary to defibrinate the blood should be employed in order to keep haemolysis to a minimum and prevent significant elevation of the serum potassium level. The blood is thereafter centrifuged, preferably in a refrigerated centrifuge (4 °C), and the serum removed by a convenient suction device. A second centrifugation is undertaken to remove the last traces of red cells.

After the pool has been thoroughly stirred, a sample is set aside for preliminary analysis, and the bulk stored in a clean container at –20 °C.

Adjustment of Analyte Concentrations

Concentrations of the various analytes can be brought to clinically useful (discriminatory) levels by undertaking adjustments.

For example, the average normal bilirubin concentration in ox serum of 0.2 mg per 100 mL (3.4 mol/L) is of little value in controlling a laboratory's performance for this test, and a value near 2 mg per 100 mL (34 mol/L) will prove more useful when sera from patients suspected of having liver disease are being examined. Similarly, a urea concentration of around 60 mg/100 mL (10.0 mmol/L) could be more

useful than the average 24 mg/100 mL (4.0 mmol/L) found in ox serum.

Bovine serum contains no measurable level of uric acid and useful concentrations can be achieved only by graded additions. It is advantageous to have stocks of quality control sera available which have been set at different levels.

With the results of a preliminary analysis of the serum pool to hand, it is possible to adjust analyte levels and offer a range of 'low', 'mid' and 'high' values for the common constituents. The actual settings of these values can be altered to suit local needs within the constraints imposed by the processes of preparation.

Material Presentation

QC sera thus prepared could be provided either in liquid state or freeze-dried (lyophilized), or could also be kept in a frozen state in the user laboratories. Where the material is to be used strictly for IQC purposes, the advantage of frozen liquid serum, distributed in suitable vials, is that accurate dispensing is not required and any error of reconstitution is obviated.

In hot climates, where sera have to be dispatched by post or other means, it is essential to use a lyophilized preparation.

Animal serum (e.g. bovine) is the preferred QC material for both IQC and EQA of most investigations in many countries because of its stability.

Frozen liquid control sera

Frozen liquid control sera are intended primarily for internal control purposes within a laboratory.

On each working day, one or more vials should be removed from the refrigerator, thawed fairly rapidly by immersing in a water bath at 25–37°C, removing the vial immediately after all the ice has melted. It should then be mixed well by gentle inversion and samples distributed to the various work benches in the laboratory so that they may be analysed in exactly the same way as patient samples. The value found for each analyte each day should be plotted on charts and statistics compiled according to the instructions[5].

Lyophilized sera

Lyophilized sera are intended for IQC purposes or use in EQASs. The volume of distilled water or reconstitution fluid used is critical. The exact volume should be stated on the label, and a special grade 1 calibrated bulb pipette (such glassware may not be readily available in developing countries) kept exclusively for this purpose. The cap should be removed slowly and carefully because particles of dried serum often adhere to the cap and must not be lost. The prescribed diluent or high quality distilled water is pipetted in, the cap replaced and the vial inverted three or four times and placed in a dark cupboard for five minutes, when it is taken out and again slowly inverted to aid solution. Vigorous shaking must be avoided. After 15 to 30 minutes, solution should be complete and the material may be used.

It is important to remember that the solid material in the vial contributes to the volume of reconstituted serum, according to its protein content. A 10 mL vial reconstituted with 5 mL of water will therefore not have exactly double the usual concentrations.

Stabilized liquid serum

Stabilized liquid serum can be extremely useful and appropriate for many developing countries. It is essential that all laboratories have reasonably large volumes of stable QC serum available. These need to be of good quality, yet cheap and easy to produce.

Of the methods available, the one chosen must utilize cheap and widely available chemicals. Some compounds

are known to invalidate enzyme assays and should not be used if the material is to be used for enzyme measurements.

Thiomersal, borate, fluoride, azide and antibiotics have all been used as preservatives, but have been found to be less satisfactory than ethylene glycol (ethanediol), as described in WHO document LAB/86.4.6. In serum containing 15% ethylene glycol, all the constituents are stable at –20 °C for at least eight months. At 4 °C, alkaline phosphatase, AST and bilirubin are stable for four months and many other constituents are stable for at least eight months.

Serum containing 15% ethylene glycol is also stable at higher temperatures for shorter periods. Initial studies indicate that all the constituents of serum are stable at 25 °C for six days and at 37 °C for three days. The stability at these temperatures is likely to be variable depending on the level of contamination by microorganisms.

Materials containing ethanediol may permanently damage electrodes and thus are not suitable for direct ISE instruments for electrolyte analysis.

Serum prepared in this way may therefore be distributed at ambient temperature for EQA programmes in addition to its use for IQC.

In India, the use of ethylene glycol at 25% has been found to provide more stablity in serum considering the weather conditions, and is being recommended for warmer tropical climates.

Matrix effects with manual methods are unlikely, though they will arise with systems including dialysis or viscosity-dependent processes.

Materials containing ethanediol are not suitable for direct ISE instruments for electrolyte analysis (i.e. those presenting undiluted serum to the electrode) as the electrode may be permanently damaged. The addition of any liquid to serum results in dilution of the concentration of all analytes.

For many analytes this is undesirable and involves the addition of several compounds to restore the concentration of some constituents. To avoid this, it is best to start by preparing a carefully mixed serum pool which is then frozen. On thawing, the top layer will contain very low concentrations of all constituents.

A volume, equivalent to 15% of the total, is removed and replaced with the same volume of ethylene glycol. It is then mixed carefully and thoroughly. At this stage, the serum may be assayed for any constituent and compounds added to elevate the concentrations of certain analytes. Full details are given in WHO document LAB/86.4.6.

Summary

Materials used for IQC and EQA must be appropriate for their purpose. In many developing countries, locally-produced animal serum from a convenient species and stabilized with ethanediol will provide suitable material in large quantity at low cost. Such availability facilitates the widespread use of effective QA procedures, and thereby contributes to the country's health care.

6 Scoring Systems in External Quality Assessment for Clinical Chemistry

Introduction—the Need for Scoring Systems

The objective of any EQAS is to stimulate inter-laboratory concordance of numerical results. Thus, participants require a clear demonstration of whether their results are in consensus, i.e. whether or not corrective action is needed.

Many participants experience difficulty in comparing their results with the 'target' data provided by the scheme, whether it is in the form of designated values, histograms, or statistical parameters classified according to method.

In addition, experience indicates that it is usually those laboratories which most need it, have the least time, inclination, and ability to devote to this task.

Some form of data reduction is therefore essential if participants are to derive maximum benefit from the scheme; this usually takes the form of a scoring system.

Classification of Scoring Systems

Scheme organizers have devised a plethora of scoring systems. However, the several basic types include:

- 'Pass/fail' systems
- Semi-quantitative systems
- Quantitative systems

Pass/fail systems

Such systems comprise assessment of each result against some criterion of acceptability. This criterion may be derived as a multiple of the observed SD or of the SD obtained by reference laboratories[7,8], or from an estimate of clinical requirements in the form of analytical goals[9] or medical need[10].

Semiquantitative systems

In these systems, results are classified on a semiquantitative scale, as, for example, in the allocation of 'points' according to how close the result approaches the designated value (DV). This gradation may be in terms of observed SDs in the initial definition of Variance Index[11] (VI) or of an arbitrary scale of clinical needs[12].

Such systems have also been applied to other less quantitative laboratory investigations.

Examples here are the system adopted by the UK NEQAS for microbiology, which classifies participants' returns as fully correct, partly correct, wrong and badly wrong on a scale of +2 to −1[13].

Quantitative systems

In this procedure, results are scored on a continuous scale. The main applications are SD differences (SDDs or 'Z scores')[14-16], the VI system[17] and systems based on cumulated estimates of bias and precision or consistency of bias[18-20]. In some of these applications, logarithmic transformation or other statistical manoeuvres are applied to the results before and/or after scoring[21].

'Hybrid' systems

Several schemes, for example that operated in the USA by the Centers for Disease Control[22], use a combination of these procedures.

Scoring as a Stimulus to Improvement by Individual Laboratories

Scoring systems are a potent means of data reduction, which assist participants in assessing their performance relative either to other laboratories or a defined or arbitrary standard. This objective applies to the individual laboratory situation.

The original intention behind the introduction of scoring systems was to make EQAS information more comprehensible to participants. Comparison of results with targets in the form of overall or

method-related means or histograms is difficult.

If a number of analytes (10 to 20 in many schemes covering general clinical chemistry) are involved the problem becomes more complex, even for single-specimen distribution. The assessment 'by eye' of the data accumulated over several distributions is yet more difficult, and anything other than a gross change in performance is effectively impossible to detect.

A scoring system makes such assessment much simpler through the cumulation of information in more readily comprehensible form. This cumulation may refer to a single analyte only, with use of results covering a period, to permit appraisal of performance relative to other participants at that time or to the individual laboratory's past performance. For the latter, the score must be independent of other participants' performance.

Appropriate scoring systems can also permit combination of data from more than one analyte, and so provide an overall assessment of the laboratory's performance. Again, appraisal can be against others or against previous performance. Such measures of 'performance at a glance' are extremely powerful distillations of EQAS information and are thus a useful managerial tool, provided the limitations discussed below are recognized. The most primitive systems give information in qualitative form, e.g. the 'pass/fail' criteria applied by licensing schemes[7,8], and thus give only the crudest reflection of performance.

The objective of the EQAS is to stimulate inter-laboratory concordance of numerical results.

More sophisticated systems such as VI scoring yield quantitative information as a numeric score, retaining the potential for easy interpretation and being susceptible to graphical presentation. These have been of great assistance in enabling laboratories to recognize the existence of suboptimal performance and in stimulating them to improve.

Most systems are based on an estimate of total error, but further refinement can also provide guidance on the type of contributory errors. Schemes usually rely on precision information gained separately through the laboratory's IQC programme to assist in interpretation (though some do assess imprecision through repeated distribution of the same specimens), and provide estimates of bias and its consistency.

A bias may be inconsistent due to poor within- or between-assay precision, but the potential presence of other contributory factors, such as nonlinearity or other concentration-dependent bias, short- or long-term accuracy changes and specimen/method interactions, usually precludes such a simplistic interpretation.

Application of a good scoring system can then provide a robust and reliable assessment of overall performance, indicating improvement or deterioration and identifying any need for improvement. It should then also assist in the resolution of any problems, indicated through provision of more detailed information for the individual analytes concerned, since different approaches are required for problems arising from, for example, erroneous calibration and nonlinearity.

To avoid unnecessary sifting through irrelevant information (and thus defeating the object of using a scoring system), the scoring system and report format should enable the participant to proceed in a step-wise manner through a hierarchy of increasingly detailed performance data, deciding at each stage whether it is necessary to proceed further.

A good scoring system provides a reliable assessment of overall performance.

Hierarchical interpretation of scoring

The most helpful scoring system will fail in its objective if the scores are not presented in such a way as to simplify the interpretation. Each participant has only limited resources (in terms of time, effort and ability) to devote to this interpretation, and experience suggests that those in most need of acting on EQA data devote, or choose to devote, the least.

An analyst receiving a report needs to make decisions on a series of questions, which are usually self-terminating when a negative answer is given:

- Q. Do I have a major overall problem?
- Q. Which analytes are contributing most to this?
- Q. Are these problems significant?
- Q. What is the source of the error in each case?

A well-designed combination of scoring system and report format can assist considerably in this process, and thus contribute to patient care, not only through stimulation of improvement where this is indicated, but also through removing the need for unnecessary investigation. The application of VI scoring (defined in Annex 3) in the UK NEQAS for Clinical Chemistry provides an example of such a system (see box).

Though detailed information must be provided by scoring systems, this sophistication may make it increasingly difficult to relate performance scores to patient care, especially where they are determined by consideration of the state of the art rather than clinical requirements.

In such contexts there is also perhaps additional need for a 'pass/fail' system, to indicate in a very simple manner whether the performance is good enough to satisfy criteria for medical decision.

Scoring in Assessment of Techniques

Scoring systems also simplify the assessment of overall concordance, though the primary objective of most is to stimulate improvements in the performance of individual participant laboratories. Average scores provide a measure derived from all results which is effectively distribution-free. This measure is also convenient to obtain and use, and likely to be robust, i.e. not too variable.

Scores also simplify the assessment of methods and other factors affecting performance through their cumulation of information over time. The resultant estimate is more representative in that it includes contributions not only from variations over time but also from differences due to analyte concentration and material distributed.

Overall performance	overall mean running variance index score (OMRVIS)
Individual analyte performance	mean running variance index score (MRVIS)
Contributory factors	mean running bias index score (MRBIS) and standard deviation of the bias index score (SDBIS). Graphs of result against designated value.

Assessment of Progress Comparisons over Time

A well-designed scoring system should delineate changes in the performance of an individual laboratory. The main requirement for this is that the score should be independent of other participants' performances. Such scoring systems also enable assessment of trends in inter-laboratory agreement, and provide a means of judging the overall success of the scheme in stimulating improvement.

Scoring systems such as the VI system can also assist in differentiation among the factors (bias and variability of bias) contributing to variance.

Geographical Comparisons

Average scores provide a convenient and reliable assessment of performance in different groups of laboratories. These may be groups within a scheme or laboratories participating in different schemes.

If participants in different schemes are to be compared, the scoring system must again be one which is independent of the performance of other participants. Otherwise the comparison is meaningless, since, for example, the average SDD in any scheme will be approximately the same and does not reflect the standard of performance within the scheme. The VI system is such a system.

A well-designed scoring system should delineate changes in the performance of an individual laboratory.

Appraisal of Scoring Systems

Having reviewed the uses of scoring, how well do the various systems which have been devised fulfil the objectives?

Assessment of imprecision

Though bias is the prime concern of EQA, with imprecision assessed primarily through IQC procedures, it may be advantageous to assess both aspects of performance since laboratories may not have (or may not take sufficient note of) an effective IQC programme to assist in interpretation of EQA data. In addition, IQC data are, by definition, internal to the participant laboratory and it would otherwise be impossible in an EQAS to reliably interpret an assessment of bias without also having some indication of within-laboratory precision as an estimate of confidence in the bias assessment.

Some EQA schemes include a formal assessment of imprecision, through the replicate analysis of specimens. This may be by 'open' replicate analysis of each individual specimen, which is obviously open to manipulation by participants, as in the 'hybrid' IQC/EQA schemes[23-25]. The alternative is repeated distribution of the same specimen as part of the scheme design, to afford an estimate of within-laboratory precision[16,18,19,26]. Some of these schemes use this estimate as their only or primary assessment of laboratory performance[16,26]. This is merely a duplication of what the laboratory's IQC programme should be providing and is hence a waste of resources. If the semi-blind EQA replicate data conflict with the IQC estimate then the more favourable figure will tend to be believed.

Standard deviation differences

More complex algorithms, such as the standard deviation difference (SDD) or 'Z score'[14,16], may be employed as indicators of laboratory performance. The SDD is calculated by dividing the observed SD into the difference of the participant's result from the mean.

This statistic, however, can only be used to compare performance of laboratories with others in the same scheme at the same time. SDDs cannot be compared over time since the performance of the other

laboratories (and hence the observed SD) may change, and with laboratories in different schemes the observed SD will almost certainly differ. The average SDD in any scheme at any time will always have a similar value, and changes will only represent a change in the shape of the frequency distribution of results.

The variance index system

Consideration of the above factors led to the devising and subsequent refinement of the Variance Index (VI) scoring system[11,17,20,27]. The formal definitions are given in Annex 3.

Variance indices offer a convenient system for comparing assessments of performance over time and between laboratories. Cumulation over analytes and over time yields an overall mean running VIS (OMRVIS) as an empirically-useful assessment of overall performance. The VIS is a mixed index, responsive to failures of both accuracy and precision, but the MRBIS and SDBIS[20] afford a means of separating the effects of these aspects of performance. They thus correspond closely in intent to the cumulative bias (BIAS) and cumulative variability of the bias (VAR) estimators used in UK NEQASs for hormone assay[28].

Variance indices offer a convenient system for comparing assessments of performance over time and between laboratories.

Variable chosen coefficient of variation systems

One assumption in the VI system is that the coefficient of variation (CV) is constant throughout the concentration 'window' used for performance assessment. In many cases this is approximately true, but in others, there is substantial concentration-dependence.

A refinement is to compensate for this by introducing a variable chosen coefficient of variation (CCV)[29], (based on previous experience in the scheme) of the relationship of inter-laboratory CV to concentration. Though this provides an excellent compensator, other potential problems are also introduced. With advances in technology or participants' performance, the relationship is likely to change; it may also differ from scheme to scheme. Adjusting the CCVs too frequently will then lead to loss of continuity in assessment, and the system will reduce almost to a 'variable SDD' situation with the consequent disadvantages discussed above.

Cumulative bias and the cumulative variability of the bias

These parameters[18,30] provide an assessment of bias and its variability, both in percentage terms. The definitions are given in Annex 4. Interpretation is similar to that of the MRBIS and SDBIS, but there are several differences.

Firstly, the scores are not in a 'common currency'. Thus the scores for analytes cannot be combined to give an estimate of overall performance.

Interpretation must also be individual for each analyte, whereas in the VI system similar performance relative to the state of the art yields similar scores. Clinical relevance may be more readily discerned from the percentage error presentation, but VISs can be transformed simply into such terms (e.g. a BIS of –85 for calcium indicates a negative bias of 3.4%).

Secondly, the derivation of cumulative bias (BIAS) and the VAR include an outlier elimination procedure. Any discrepant individual bias is classified separately as a 'blunder' and is therefore not lost entirely from consideration, but does not contribute to the performance assessment used most commonly in these schemes. In the VI system, the effects of grossly discrepant individual

results are mitigated by truncation of the VIS at a maximum of 400, but such scores do still contribute.

Competition in External Quality Assessment

The competitive instinct was cited above as one factor motivating attempts to improve performance, and scoring facilitates competition in EQA, particularly when it includes a 'league table' presentation of scores. Anything which improves comparability of results should be welcomed as benefiting patient care, but care must be exercised in exploiting this urge.

Firstly, it may encourage an attitude of improvement for improvement's sake, irrespective of clinical requirements, so that attaining good performance in EQASs becomes an end in itself.

More disturbingly, this may lead to a dissociation between the procedures used for EQA specimens and clinical specimens. Thus, if assay replication and other favourable treatments[31,32] are used, EQAS performance will no longer be an objective reflection of that normally attained in the laboratory. This is fundamentally dishonest, though such 'cheating' only deceives the participant laboratory into a false impression of the reliability of its assays.

The Variance Index (VI) scoring provides a simple and reliable indication of laboratory performance and changes over time.

For these reasons, most scheme organizers try to avoid an excessive competitive element while still encouraging a healthy striving to emulate the performance of the best laboratories. For example, presentation of OMRVISs in a league table format in the UK NEQAS for general clinical chemistry was discontinued in the late 1970s because some participants were reportedly more concerned with their position than with the OMRVIS itself.

The Basis of Assessment: State-of-the-Art or Clinical Requirements?

The relative merits of assessing laboratory performance against attainable standards or medical needs have always generated controversy. In the early years, EQA against the state of the art was the only feasible procedure, tempered later by consideration of the effects of any errors on clinical care.

Indeed, there had been no realistic estimate of clinical requirements, apart from Tonks' criterion of errors not to exceed one quarter of the 'normal range', as the reference interval was then called[33], and various statements derived from subjective or even arbitrary views of individual clinicians[34], as discussed by Fraser[35].

Against this background, many scoring systems were established using attainable performance as a baseline. For example, scoring in terms of SDDs or 'Z scores' had been widely practised in many surveys as a way of compensating for differences in interlaboratory agreement at varying analyte levels[15,16]. This also enabled expression in terms of a 'common currency' for all analytes, with similar performance relative to other laboratories giving scores of similar magnitude.

In Germany, the concept was amended to define limits of acceptability in terms of the SD obtained by reference laboratories, though the effect was similar[8].

SDDs, however, remained dependent upon the general standard of performance, and an advance was required. This came with the adoption of the best interlaboratory trimmed CVs attained in the UK in 1972 as the Chosen Coefficients of Variation (CCVs) in the VI system[27]. The objective was to scale against analyte level and

analyte performance in deriving an index of overall performance which could be used as an indicator of changes over time. Other schemes chose systems related in some way to perceived clinical needs.

Thus the Netherlands scheme[12] assessed deviations from the designated value (DV) in terms of a points scale, with, for example, the same percentage deviation giving a worse score for calcium than for urate. The principal disadvantage of this type of system is that it precludes combination of the scores obtained for the analytes surveyed unless all participants offer the same range of analytes. Otherwise a laboratory could artificially 'improve' their assessment by refraining from reporting calcium results, which would score poorly.

In recent years, more objective analytical goals for imprecision and total laboratory error have been established for many commonly-determined analytes, in relation to biological variation[35,36]. These have been adopted as criteria of acceptability in some EQASs, such as that in Australia[19], giving performance standards which should be realistic estimates of clinical requirements.

Performance criteria of similar derivation have also been proposed for use in Germany[10], and have recently been endorsed. These introductions have largely been in schemes using 'pass/fail' criteria, for licensing or educational purposes. Since analytical goals are not at present met for most analytes, these goals have not been implemented in their entirety or almost all laboratories would fail. This entails a further assumption of the level of performance which is acceptable.

The quantitative performance information supplied through such schemes is rather limited in most cases, so the main emphasis in performance assessment remains on scoring systems based on the state of the art. The problem of combining scores for different analytes remains, irrespective of the advances in derivation of medical needs.

With the availability of analytical goals, state-of-the-art systems can now be related to clinical requirements. Thus, for example, the goal for calcium of 0.8% can be combined with the CCV of 4.0% to yield an acceptability criterion of 20 VIS. Comparison of this figure with the average MRVIS of 67 for calcium in December 1986 in the UK NEQAS for clinical chemistry emphasizes the problems entailed by the use of medical needs in EQA. Among the non-enzyme analytes, average performance satisfies such goals only for potassium, urea, iron and bilirubin.

With the availability of analytical goals, state-of-the-art systems can now be related to clinical requirements in many countries because of its stability.

For urea and bilirubin, however, clinical situations such as the detection of changes at elevated concentrations may make more stringent demands than the biological variation in normal subjects upon which these goals are primarily based. Similar arguments apply also to enzymes such as ALT and CK, for which the derived 'goals' of 19.7% and 35.8% CV appear intuitively too permissive.

Given this continuing controversy, which basis is better? The choice depends on the purpose of the assessment, with state-of-the-art assessment being more convenient and versatile in most EQASs, and clinical requirements providing a more objective appraisal of the extent to which the results of laboratory investigations can fulfil their potential in patient care. On balance, the best approach seems to be the use of a system such as VI for 'routine' application, with analytical goals being used to assist rational choice where particular decisions (such as the relative need for improvement of two analytes) have to be made.

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Annex 3

Variance Index Scoring System

General Aspects of Variance Index (VI) Scoring

Variance Index scoring was described originally by Whitehead et al. (1973)[11]. This was soon changed to the more reliable version incorporating scaling by chosen coefficient of variation (CCV) rather than by SD[17,27]. Later refinements which introduced BISs and their cumulated running indices were described by Bullock and Wilde (1985)[20]. The system provides a simple but reliable indication of laboratory performance which has proved useful over many years in assessing not only laboratory performance but also of changes in this over time.

Not all specimens are included in the performance assessment, which in general excludes those with very high or very low analyte concentration.

VI Scoring in EQASs

The definitions and derivation of the parameters used are as follows:

Bias Index Score (BIS)

This score is the difference between the result obtained by the laboratory (x) and the designated value (DV—see below) expressed as a percentage of the method mean, divided by the CCV (see below) for the analyte and again expressed as a percentage:

$$\text{BIS} \qquad \frac{(x - DV)}{DV} \; \frac{100}{CCV}$$

Any score greater in magnitude than 400 is set to 400. The BIS may therefore be in the range –400 to +400.

Designated Value (DV)

The DV is the 'target value' for the analyte in the specimen distributed. It is usually an overall or method-related mean (consensus value) from the scheme, recalculated after exclusion of outliers, preferably detected using the system of Healy (1979)[21] as a robust procedure.

Chosen Coefficient of Variation (CCV)

The CCV is a scaling factor for each analyte, correcting for differences in the state-of-the-art and yielding VISs in a 'common currency'. It does not represent a 'clinically acceptable error'. For the original[14] general clinical chemistry analytes, CCVs are the best interlaboratory CVs achieved in the UK NEQAS in 1972, which are still representative of the relative performance. CCVs for other analytes were selected to yield similar VISs, often using 'calibrated' data for reliable method groups[36].

Variance Index Score (VIS)

The VIS is the absolute value of the BIS, i.e. ignoring its sign. Values may be in the range 0 to 400.

Mean Running VIS (MRVIS)

The MRVIS is the mean of the ten most recent VISs for the individual analyte, or of all VISs for the analyte from the six most recent distributions, where distributions comprise more than one specimen. Values may be in the range 0 to 400.

Mean Running BIS (MRBIS)

The MRBIS is the mean of the ten most recent BISs for the individual analyte, or of all VISs for the analyte from the six most recent distributions, where distributions comprise more than one specimen. Values may be in the range –400 to +400.

Standard Deviation of the BIS (SDBIS)

The SDBIS is the standard deviation of the ten most recent BISs for the individual analyte, or of all VISs for the analyte from the six most recent distributions, where distributions comprise more than one specimen. Values may be in the range 0 to 422.

Overall Mean Running VIS (OMRVIS)

The OMRVIS is the mean of all VISs for the laboratory from the six most recent distributions irrespective of analyte. Values may be in the range 0 to 400.

Graphical Presentation of VIS Data

Graphs of running scores against time are produced, covering a period of about two-and-a-half years for a scheme with two-weekly distributions. These are plotted against distribution number and include OMRVIS alone, MRVIS alone, or MRVIS, MRBIS and SDBIS together. Indications of the current 5th, 50th and 95th centiles of OMRVIS or MRVIS are included.

Annex 4
BIS/VAR Scoring System

General Aspects

These scores are used in all UK NEQASs for hormone assay, where they have proved useful for performance assessment[18]. They provide an assessment of bias and its variability, both in percentage terms.

Not all specimens are necessarily included in the cumulative assessment, which in general may exclude those with very high or very low analyte concentrations. Where there are 4–6 specimens in each monthly distribution, a 6-month cumulation period is used.

BIAS/VAR Scoring in EQASs

The definitions and derivation of the parameters used are as follows:

Bias

The percentage bias of a participant's result relative to the DV.

Designated Value (DV)

The DV is the 'target value' for the analyte in the specimen distributed. It is usually an overall, method group-related or method-related mean (consensus value) from the scheme, recalculated after exclusion of outliers, preferably detected using a robust procedure such as the system of Healy (1979)[21]. In some situations, results may be expressed as logarithms before data processing, yielding a geometric rather than an arithmetic mean.

Cumulative Bias (BIAS)

The BIAS is the arithmetic mean of the Healy-trimmed bias values for all usable specimens in the six most recent distributions (e.g. for up to 30 specimens if there are five specimens per distribution). Where results have been transformed logarithmically, the BIAS is the corresponding geometric mean.

Cumulative Variability of the Bias (VAR)

The VAR is the standard deviation of the Healy-trimmed bias values for all usable specimens in the six most recent distributions. Where results have been transformed logarithmically, the VAR is the corresponding geometric CV.

Chapter 3

Quality Assurance in Microbiology

1. Introduction

QUALITY ASSURANCE (QA) in clinical microbiology is particularly important in the South-East Asia Region where infectious diseases are still the leading cause of morbidity and mortality.

Implementation of a QA programme is less easy in microbiology than in the other branches of laboratory medicine. Test results in microbiology are generally non-numerical and qualitative. The interpretation of test results may be rather subjective and should be based on good communication with the clinician.

The laboratory investigation of an infected patient is rather complex, involves several steps, and is less standardized than in other laboratory disciplines.

Internal quality control (IQC) in microbiology must be comprehensive and cover every step in the cycle, from the collection of the specimen to sending the report to the clinician. Since specimens from infected patients are not homogeneous and stable, they cannot be used as such in external quality assessment schemes (EQAS). They are replaced by cultures which are immediately recognized as EQAS material by the laboratory staff, who give these cultures better treatment than routine cultures.

It is important to realize that the need for quality is not limited to the actual microbiological investigations conducted in the laboratory.

Errors at any stage in the investigation can affect the outcome. The right patient must be identified, relevant tests ordered and appropriate specimens obtained, transported to the laboratory in an unchanged state and appropriately labelled with laboratory accession codes on receipt.

Following technical analysis, results must be interpreted by the laboratory personnel, and the report must maintain the link between the specimens taken and the patient from whom they were obtained. The format and quality of the laboratory report may well affect the interpretation by the clinician, which will in turn affect diagnosis and treatment. Obviously, errors can occur at each stage of the chain and the QA programme must seek to minimize this. Although some of these factors may be outside the laboratory's control, a process of monitoring can help to identify problems, and liaison with clinical, nursing and portering staff can help to correct them.

Internal quality control in microbiology needs to be comprehensive, from collection of specimens to sending the report to the clinician.

2. Internal Quality Control (IQC)

Quality of Specimens

SPECIMEN QUALITY deserves special attention in microbiology. It is common to receive samples which are labelled 'sputum' but are obviously saliva. Similarly, a badly taken urine sample will allow contamination with indigenous flora and thus nullify the results of quantitative bacteriology.

Clinicians and nursing staff should be provided with written instructions on what specimens are appropriate and how they should be collected. The continual reinforcement of written protocols through discussion with clinical colleagues when inappropriate specimens are received, will help to maintain cooperation.

Internal Auditing of Samples

Senior staff within the laboratory may divide a proportion of incoming specimens into two parts, one of which will be processed as the patient's specimen, and the other as a known 'QC' specimen. The laboratory worker examining the specimens will not know which patients' specimens are the same as 'QC' specimens. Comparison of the results obtained will provide valuable information on the reproducibility of results within the laboratory.

Experience with EQASs suggests that transposition errors are not uncommon. In cases where the results of tests are likely to have a significant effect on subsequent treatment, such as the demonstration of acid- and alcohol-fast bacilli (AAFB) in sputum, and where resources allow, re-examination of the specimen from the original container may be useful.

In cases where the results are likely to have a profound effect on the patient, as with a positive test for HIV antibodies, collection of a repeat specimen from the patient is a good practice.

Clinicians and nursing staff should be provided with the standard operative procedure on collection and transportation of samples.

Monitoring of Equipment, Materials and Procedures

Equipment

Regular checks should be made to ensure that the equipment is performing within specifications. The temperature of incubators, water-baths, refrigerators and freezers must be monitored daily and the results recorded on simple charts which can be displayed on the equipment. The sterilizing cycles of autoclaves and hot air ovens must be monitored and the results recorded, to ensure that adequate temperatures are maintained for sufficient time.

Spores of *Bacillus stearothermophilus* are traditionally used for testing autoclaves. However, with this method, failure of an autoclave cycle is only revealed several days after the event, which is usually too late to prevent adverse consequences. Many microbiologists now rely on the use of thermocouples for measuring the temperature to control autoclave cycles.

Exhaust protective cabinets must be checked regularly to ensure that adequate airflow is maintained. A planned, preventive maintenance programme must be introduced for all equipment. The frequency and

complexity of checking and maintenance will vary according to the equipment, with robust or uncomplicated equipment needing less frequent checks than delicate or complex equipment.

Quality-related procedures require intelligent questioning of need and resources rather than blind implementation; discrepancies must be investigated and causes corrected.

Microscopes must be regularly cleaned and checked to ensure accurate optical alignment. Diluters and pipettes should be regularly checked by gravimetry or by the calibration kits supplied by the manufacturer.

Splashing and cross-contamination must be avoided during dispensing. Modern serological tests are so sensitive that even minimum cross-contamination may lead to false-positive results.

Plate washers used in serological tests such as ELISA are prone to blockage due to accumulation of salts precipitated from buffers. This must be avoided by regular flushing with water as blockage may result in uneven washing of plates. This can be checked by placing diluted dye solutions in each well of a plate before washing.

Avoid splashing and cross-contamination since modern serological tests (ELISA) are sensitive enough to give false-positive results with minimum cross-contamination.

Unevenness of washing is indicated by retention of the dye solution in some wells. If unevenness of washing proves to be an intractable problem, then rotating the plate through 180° half-way through the washing cycle will usually compensate. Readers should be checked regularly with blank plates to ensure evenness of background reading and with dilute dye solution to ensure that all wells are read.

Water

The purity of distilled or deionized water should be checked regularly by measuring its conductivity. Visual inspection or chemical analysis must be done to check for organic contamination.

A planned, preventive maintenance programme must be introduced for all equipment.

Disinfectants

The preparation of disinfectants must be monitored to ensure that they are appropriately diluted, and containers must be checked to ensure that they are not overloaded with organic matter. Discarded material must be adequately immersed in disinfectants.

Culture media

Each batch of culture medium should be checked with control strains before use to ensure that it supports the growth of bacteria and, in the case of selective media, inhibits the growth of non-pathogens.

An economical approach to QC of media and reagents has been suggested[1], and the need for frequent, repeated QC of media and reagents that are known to be trouble-free and reliable has been questioned. Efforts can thus be concentrated on those culture media which are known to present problems. It is, however, necessary for the user to identify the reliable and unreliable media and reagents before QC schedules can be established and periodically reviewed.

A two-tier system of QC testing of media has been suggested by Martin[2], in which extended panels of organisms are used to assess media from new suppliers, new products, comparability between suppliers,

and new batches from the supplier. A smaller panel is used to assess routine batches of media. This system makes use of standard strains available from the UK National Collection of Type Cultures (NCTC) or the American Type Culture Collection (ATCC).

The properties to be controlled are the ability to support growth from small inocula of the organisms sought and, in the case of selective media, ability to suppress the growth of large numbers of commensal organisms.

Strains of the same species may differ in their growth characteristics and in their sensitivity to selective agents. Sensitivity of media is best controlled by strains that are particularly fastidious or sensitive to selective agents, and specificity of media by the use of strains that are particularly resistant to selective agents. The use of such strains will ensure that media that perform well with them are also likely to perform well with most organisms met in routine specimens. Such strains can be selected by careful observation of the growth characteristics of bacteria isolated from the laboratory's workload.

Control strains often tend to lose their characters and become adapted to laboratory media on storage, especially if frequently sub-cultured.

Control strains must be stocked in aliquots since they tend to lose their characters when frequently sub-cultured.

Regular replacement, or use of a different preservation method may therefore be necessary. Inoculum size is important in QC testing.

A relatively poor medium may support a heavy inoculum of a pathogen but fail to support the lower numbers often found in clinical specimens. The inoculum of control strains should, therefore, be diluted appropriately before application.

Characterization tests

Characterization test reagents, such as those used in the detection of carbohydrate fermentation, should each be tested after preparation or on receipt of a newly manufactured batch, by using two strains, one of which produces positive results and the other negative results.

By a careful combination of strains, a large number of tests can be controlled with few strains[3] (see Tables 1 and 2). Such control procedures are particularly useful in circumstances where it is suspected that commercially produced identification systems are affected adversely by transportation or storage. In addition to providing a check on the performance of tests, the use of such controls acts as a useful teaching aid by allowing staff to gain experience of positive and negative results.

Gram stain

Perhaps one of the cheapest and most effective IQC measures is the routine use of the Gram stain on clinical material.

Microscopic examination provides information on the quality of the specimen, e.g. it will distinguish between sputum and saliva. With experience and training, it is possible to identify with some degree of accuracy the microbiological flora present.

A comparison of the results of subsequent cultures with the list of organisms seen on microscopy will provide a useful check on the efficacy of culture. New batches of stains, such as Gram and Giemsa should be checked with known positive and negative specimens.

A comparison of the results of subsequent cultures with the list of organisms seen on microscopy will provide a useful check on the efficacy of culture.

Table 1

NCTC control strains for commonly-used tests

Test	NCTC number	
	Positive	Negative
Aesculin hydrolysis	11935	11934
Citrate utilization	7475	11934
Decarboxylases		
arginine	11936	7475
lysine	11935	7475
ornithine	11935	7475
Deoxyribonuclease	11935	11934
Gelatin liquefaction	11935	11936
Gluconate oxidation	11936	7475
Hydrogen sulphide (TSI)	11934	7475
Indole production	7475	11935
KCN tolerance	7475	11934
Malconate utilization	11936	7475
Methyl red	7475	11935
ONPG	11935	7475
PPA production	7475	11936
Selenite reduction	11936	11934
Urease	7475	11935
Voges Proskauer	11935	7475
Gas from glucose	11936	11935
Acid from sugars		
Adonitol	7475	11934
Arabinose	11936	11934
Dulcitol	11936	11934
Inositol	11935	11934
Lactose	11936	11934
Maltose	11936	7475
Mannitol	11936	11934
Raffinose	11936	11934
Rhamnose	11936	11934
Salicin	11935	11934
Sorbitol	11936	11934
Sucrose	11936	11934
Trehalose	11936	11934
Xylose	11936	11934

11935 Serratia marcescens, 11934 - Edwardsiella tarda, 7475 - Proteus rettgeri, 11936 - Enterobacter cloacae

Antimicrobial susceptibility testing

Disc testing is the most commonly used method for testing susceptibility. The main sub-methods used are various refinements of the Kirby–Bauer technique, of which the NCCLS method is the most common, and comparative methods, exemplified by the Stokes' method. With Stokes' method, the control is an integral part of the test and is inoculated on to the same plate as the test strain. The original method described the use of three control organisms: the Oxford strain of *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418) and *Pseudomonas aeruginosa* (NCTC 10662).

These strains are, however, not suitable for testing all species with all antibiotics, and in some cases, the use of controls belonging to the same species as the organism being tested appears to be beneficial[4].

In the NCCLS method, controls are tested on a separate plate, at a frequency dictated by a statistical evaluation of results obtained previously with controls. The disc content is critical in the NCCLS method, and the results obtained are valid only if controls are used exactly as specified. This may be particularly critical in climatic conditions which are likely to result in deterioration of discs.

Table 2

Further commonly-used tests and suggested controls

Test	Control organisms	NCTC numbers	Expected results
Bacitracin	*Streptococcus pyogenes*	8198	Sensitive
	Streptococcus viridans	10712	Resistant
Catalase	*Staphylococcus aureus*	8532	Positive
	Streptococcus pyogenes	8198	Negative
Coagulase	*Staphylococcus aureus*	8532	Positive
	Staphylococcus epidermidis	4276	Negative
Deoxyribonuclease	*Staphylococcus aureus*	8532	Positive
	Staphylococcus epidermidis	4276	Negative
Haemolysis	*Streptococcus pyogenes*	8198	β-haemolysis
	Streptococcus viridans	10712	α-haemolysis
	Streptococcus epidermidis	4276	No haemolysis
Oxidation/ fermentation	Pseudomonas aeruginosa	10662	Positive
	Serratia marcescens	11935	Fermentative
	Acinetobacter lwoffi	5866	Alkaline or negative
Motility	*Serratia marcescens*	11935	Motile
	Acinetobacter lwoffi	5866	Non-motile
Nitrate reduction	*Serratia marcescens*	11935	Positive
	Acinetobacter lwoffi	5866	Negative
Optochin sensitivity	*Streptococcus pneumoniae*	10319	Sensitive
	Streptococcus viridans	10712	Resistant
Oxidase	*Pseudomonas aeruginosa*	10662	Positive
	Acinetobacter lwoffi	5866	Negative
Phosphatase	*Staphylococcus aureus*	8532	Positive
	Staphylococcus epidermidis	4276	Negative
Toxigenicity testing of *C. diphtheriae*	*Corynebacterium diphtheriae*	10648	Positive
	C. diphtheriae	3984	Weak positive
	C. diphtheriae	10356	Negative
X and V factor	*Haemophilus influenzae*	10479	Requires X and V
	H. parainfluenzae	10665	Requires V
	H. canis	8540	Requires X

Whichever method is used, it is important to be sure that the control organisms are those intended and have not been replaced by overgrowth of the cultures with contaminants. To avoid this possibility, working controls normally stored on slopes should be replaced at weekly intervals from stock cultures stored by a more secure method such as gelatin discs.

Anaerobic growth

To check that anaerobic conditions are attained, controls must be included whenever an anaerobic jar is set up. Growth of a control strain of *Clostridium tetani*, which can be maintained in cooked meat broth, is used as a control.

The sensitivity of *Clostridium perfringens* to metronidazole has recently been shown to be an extremely sensitive indicator of anaerobiosis. The spores of *C. perfringens* in alcohol are also suitable for this purpose and can be stored for long periods[5].

The principles underlying the control of serological tests have been described by Harrison and Malic[6]. Agglutinating antisera used for the identification of microorganisms such as salmonellae can be tested on receipt of a batch or after prolonged storage by the use of heated suspensions of control strains stabilized by formaldehyde and can be stored in the refrigerator[7].

It is important to ensure that control organisms are replaced at weekly intervals from stock cultures to ensure purity of cultures.

Tests which demonstrate the presence of antibodies in the serum of patients should be controlled by including one or more positive controls in each batch. Although the manufacturers of diagnostic assays usually provide a kit control, these are designed to control individual batches and do not provide continuity of control between batches.

Use of an independent control allows comparison between batches and provides assurance of the reliability of the product. Control sera can either be prepared centrally and distributed to laboratories or prepared locally.

Use of a national control serum provides valuable standardization and is particulary desirable where national programmes, such as AIDS control, are being implemented. The controls become even more useful if information on results obtained is collated; this can be achieved by issuing a questionnaire with each control serum issued. Where a national control serum is not available, locally produced controls can be used.

A positive serum is diluted to give appropriate test readings and aliquots are stored and used as controls with each batch of tests performed. Results obtained can be recorded on charts and simple statistical analysis provides decision limits for acceptance or rejection of batch results. Details of preparation and use of such controls have been described[8].

Monitoring of equipment and materials will be ineffective unless the results are assessed and appropriate action taken. The responsibility for such assessment and subsequent action must be clearly assigned, and senior staff must periodically audit such activities.

Locally prepared control sera are valuable with regard to standardization and are desirable in national programmes, such as AIDS control.

3 Internal Quality Control Materials

Bacteriology

PERIPHERAL LABORATORIES should keep an assortment of unstained positive smears which can be used for IQC, particularly when there is any change in procedure, or when a new batch of stain or other reagent is used. In intermediate and central laboratories, one important aspect of IQC is the acquisition and maintenance of stock cultures which are used for monitoring the performance of commonly used culture media and diagnostic reagents, and for the weekly quality control of routine antibiotic susceptibility tests. The following control strains are recommended by WHO for the Kirby–Bauer disc diffusion test: *Staphylococcus aureus*, ATCC 25923; *Escherichia coli*, ATCC 25922; *Pseudomonas aeruginosa*, ATCC 27859.

IQC cultures should be selected so that the maximum morphological, metabolic and serological characteristics can be tested with a minimal collection of strains.

For a list of suggested stock strains, maintenance procedures, performance tests on media and reagents, and zone diameter control limits in the Kirby–Bauer disc diffusion test, the reader is referred to the following WHO manuals:

1. Vandepitte et al. *Basic laboratory procedures in clinical bacteriology*. Geneva, World Health Organization, 1991.
2. El-Nageh et al. *Basics of quality assurance for intermediate and peripheral laboratories*. Alexandria, World Health Organization, Regional Office for the Eastern Mediterranean, 1992.

Parasitology

Unstained blood smears of patients with malaria are used to monitor the quality of new batches of stain and buffer. Similarly, a reference collection of positive stool specimens, preserved in 10% formalin, should be used for the IQC of the staining (*Cryptosporidium*) and concentration procedures.

A collection of reference specimens is also an invaluable tool for training the junior staff in identification of unusual parasites.

Stock cultures for IQC can be obtained from

- Official culture collections: American Type Culture Collection (ATCC), National Collection Type Cultures, UK (NCTC)[9]
- Commercial producers (Bactrol R Difco)
- EQAS surveys
- Reference laboratories
- Documented isolates from clinical specimens.

4 External Quality Assessment in Microbiology

Selection of External Quality Assessment Specimens for Distribution

IN MOST COUNTRIES, financial and logistic constraints prevent the initial implementation of a comprehensive scheme for microbiology and some selection will be necessary. The decision as to which type of specimens should be included in QA distributions will depend on a number of factors which may be unique to individual countries. Some suggestions for the types of specimens that could be distributed

to different levels of laboratories are shown in Table 1. The following considerations should influence the decision.

Public health and clinical relevance

QA should initially address those situations where the quality of laboratory testing is most likely to affect diagnosis and treatment of diseases of major public health or clinical significance.

Expense

The cost of preparing reliable QA specimens should be taken into account. Specimens such as blood films or sputum slides, are relatively inexpensive to prepare. Serological specimens, however, may require extensive predistribution treatment and testing with commercially produced diagnostic assays and will be much more expensive.

Availability of material

Sufficient quantities of material necessary for the production of specimens must be locally available.

Ease of preparation

When initiating a QA scheme, it is wise to start with specimens that are easy to prepare and do not present great technical challenges. Examples of specimens which fulfil these criteria are blood slides for diagnosis of malaria and smears for examination for AAFB.

In most countries, financial and logistic constraints prevent the initial implementation of a comprehensive QA scheme for microbiology.

Analyses

Bacteriology

In peripheral laboratories, bacteriological examinations are limited to direct microscopy of sputum for acid-fast bacilli (AFB), urethral pus for gonococci, and urine sediment for Gram-negative rods.

In intermediate and central laboratories, diagnostic activities also include:

- Isolation and identification of organisms from different types of specimens, often containing one or more pathogens associated with a mixed commensal flora.
- Antibiotic susceptibility testing of clinically relevant bacteria.
- Immunological tests, such as antigen detection tests (meningitis) and antibody tests for infections including typhoid fever, brucellosis, syphilis, streptococcal infection and HIV.

Viral immunology

QA of testing methodology of bloodborne viral infections, especially testing for HIV antibody, HBs antigen and HBV and HCV antibodies is essential. It is suggested that the same organizer conducts EQAS for both microbiology and haematology.

Parasitology

Parasitological examinations are fundamentally the same at all laboratory levels. They include the examination of stained blood smears for malarial parasites, trypanosomes and microfilariae. On request, other body fluids should also be examined, CSF and lymph node aspirate for trypanosomes or urine and skin snips for microfilariae.

Shipment of Specimens for External Quality Assessment Schemes in Microbiology

According to postal regulations, the packaging must be in three layers. The primary containers (tubes, ampoules or vials with organisms) should be wrapped in sufficient absorbent material (paper, cotton) to absorb the contents in case of leakage or breakage. They should be enclosed in a rigid secondary container, which in turn is packed in an outer shipping container made of cardboard or other shock-resistant material.

The outer package should be clearly labelled 'Urgent biological

specimen for laboratory analysis'. Shipping specimens internationally still remains a major problem for which a practical solution is urgently required[13].

Assessment of Performance

A scoring scheme is valuable in providing a quantitative measure of performance. This allows comparison between laboratories, thus allowing participants to evaluate their performance in relation to that of their peers.

In the United Kingdom scheme, a four-point scoring system is used for most specimens, with a score of two awarded for a fully correct response, one for a partially correct response, zero for an incorrect result and minus one for a grossly misleading result.

The scheme can easily be adapted to reflect local practices and expected standards by altering the level of report which attracts a full or partial score. The scheme can also be modified for use in the analysis of quantitative results (e.g. titres reported) by grouping reported results into bands before scoring.

A slightly different three-point rating system is used by the WHO-sponsored IEQAS in clinical microbiology. For the identification of unknown organisms, a score of two is awarded for a correct answer, one for a partially correct answer, and zero for a totally wrong answer or failure to answer.

For antibiotic susceptibility testing, a distinction is made between a minor (one or two point) and a major (zero point) error. A minor error is when a resistant (R) or susceptible (S) organism is reported as intermediate (I) or vice versa, while a major error is the substitution of R for S or vice-versa. A score of one is given for the correct result.

The method of presenting performance data to participants used by the UK Microbiology Scheme has been reported elsewhere[14]. Computer printouts are provided to each participant after every distribution.

The printouts list the intended result for each current specimen and the participant's result and score awarded. Also shown are the participant's cumulative score obtained with previous specimens over a period of time (usually six or twelve months), the average national score obtained with these specimens and the number of standard errors that the individual participant's cumulative score is above or below the average score.

Summaries of results from all laboratories examining the current distribution, showing the various categories of reports made and the scores obtained are supplied with the individual printouts. Educational comment and teaching material are added as appropriate.

Individual printouts can also be provided periodically to show performance in a series of similar specimens, if necessary going back over a five-year period. For example, laboratories experiencing problems with a current specimen containing *Corynebacterium diphtheriae* can be presented with their results for all such specimens over a five-year period, to allow them to judge if their problem is a persistent one and to take appropriate action as necessary.

The experience in the UK scheme indicates that most participants achieve a reasonable level of performance, and that, where poor performance is noted, laboratories

A scoring scheme such as the four-point UK scheme or the WHO three-point rating scheme provides a quantitative measure of performance.

usually succeed in improving results by their own efforts. However, in some cases, poor performance persists over longer periods.

The results of such laboratories are reported to an advisory panel comprising nominees of appropriate professional societies. The function of the panel is to help the participants experiencing persistent problems in their performance in EQA by offering advice and support, as appropriate, under conditions of strict confidentiality.

Demonstrating Improvements in Performance

Since one of the essential functions of QA is to improve performance over a period of time, a means of demonstrating such improvement is needed. Trends in performance in microbiology are somewhat more difficult to demonstrate than in some other, since specimens may differ in difficulty, by design or by accident, according to the mixture of microbes included, the absolute and relative numbers of commensals and pathogens, and their growth characteristics.

As the 'state-of-the-art' improves, more challenging specimens may be distributed. Another confounding factor is that, though participants tend to improve performance with time in the scheme, the continuous addition of new participants as the scheme grows tends to hide improvements. Improvements in performance may thus appear to be slower and more irregular than would be hoped, but trends should be seen over time.

5. External Quality Assessment Scheme Materials

Peripheral Laboratories

THE MATERIAL for EQAS should include:

- Stained or unstained smears for identification of parasites (species, stage, quantity).
- Stained or unstained sputum smears for AAFB.
- Faecal specimens (1–2 mL in screw-capped vials) in 10% formalin for identification of helminth ova and protozoan cysts.

Negative specimens should occasionally be included. Specimens from patients are preferable to material from experimental animals. Smears and suspensions should be freshly prepared to avoid distortion of diagnostic morphology due to ageing. The request form should give elementary patient information (symptoms, residence, history of travel) that may be useful for guiding the examiner.

Intermediate and Central Laboratories

Most EQASs at this level concentrate on isolation, identification and susceptibility testing of bacteria and fungi (yeasts). Pure or mixed cultures are used as a substitute for patient specimens. Pure cultures are adequate to simulate a specimen of normally sterile body fluids (blood, CSF, urine), while mixed cultures of a pathogen with one or more commensals simulate an infection in a part of the body colonized with commensal bacteria (throat swab, sputum, faeces). An example of such a 'simulated stool specimen' is an artificial mixture of a *Shigella* sp. with *Escherichia coli* and a *Citrobacter* sp.

Simulated throat or stool specimens may also be used to test the ability to recognize 'negative' specimens.

The judicious selection of cultures for EQA is a delicate task. Most cultures used for surveys belong to the following categories:

- Bacteria with great public health potential, but which are rarely encountered in routine practice, e.g. *Corynebacterium diphtheriae*. Dangerous pathogens should be avoided as their use in EQA has been complicated by laboratory infections (*Salmonella typhi*, *Brucella*).
- Abnormal biotypes of common bacteria which are often misidentified, e.g. H_2S positive *E. coli*, aerogenic *Shigella*.
- 'New', newly recognized, or recently renamed bacteria, such as *Acinetobacter*, *Vibrio fluvialis*, *Xanthomonas maltophilia*.
- Familiar pathogens with a special antibiotic resistance profile, e.g. methicillin-resistant *S. aureus* (MRSA), β-lactamase producing *N. gonorrohoeae*.

Instructions and a report form (in duplicate) should accompany each survey. To make the EQA more relevant, a brief clinical statement should be included, as would be the case in a properly completed request form.

In some schemes, participants are also invited to give details of phenotypic features used for identification of unknown specimens, the method of susceptibility testing and the commercial origin of the discs. The deadline for reporting should be short (two weeks after receipt) and clearly stated.

Strains used for control purposes must be preserved in the laboratory to retain characteristics, such as viability, purity and identity.

Control Materials

Microorganisms

Strains used for control purposes must be preserved in the laboratory to retain characteristics, such as viability, purity and identity.

The relative merits of commonly used preservation methods are given below. Full technical details, source references and further discussion on all these methods have been given by Kirsop and Doyle[9].

Serial subculture and storage on nutrient media

The advantages of this method are low cost and simplicity and that no expensive reagents or equipment are required.

There are many disadvantages. Survival is uncertain, and although some microorganisms such as staphylococci and enterobacteria may survive for years, more fastidious organisms may have a shelf-life measurable only in weeks. High storage temperatures decrease survival times. Drying of media due to imperfect sealing is a common cause of loss of cultures. The period between subculture can be increased for some organisms by storing these at 5 °C in tightly closed screw-capped containers. Contamination is a constant risk. Strains may alter their characters after repeated subculture. The risk of mislabelling is multiplied with frequent subculture.

Storage in freezers

Freezing suspensions of microorganisms in a cryoprotectant such as glycerol followed by storage at low temperatures has proved successful for many microorganisms. A useful variant is freezing the suspensions on to glass beads, which can be removed individually from storage containers, thus permitting subculture without thawing a whole batch.

Advantages of this method

Smears and suspensions should be freshly prepared and fixed to avoid distortion of morphology due to ageing.

include relatively long shelf-life, freedom from contamination, stability of characters and relatively little need for manipulation.

The main disadvantages are the high capital cost of a freezer and the need for an uninterrupted power supply. Unfortunately, preservation becomes much more reliable with lower temperatures.

Both theoretically and in practice, storage at around –20 °C is often unsatisfactory since hardy organisms survive but delicate ones quickly become nonviable. Storage at –70 °C is generally satisfactory, but freezers which operate at this temperature are very expensive and prone to break down in tropical conditions.

Storage in liquid nitrogen

This method offers the advantages of almost indefinite shelf-life, freedom from contamination, stability of characters and relatively little need for manipulation.

Disadvantages include a moderate capital investment in the equipment and a contant supply of liquid nitrogen is required which is expensive. Failure in the supply of nitrogen can cause the loss of a complete collection.

This method is likely to be available only in central reference laboratories where it can be used to store secure stocks of important cultures from which working cultures can be prepared and preserved by the other methods described here.

To retain the viability, purity and identity of control strains these should be preserved in the laboratory using standard preservation techniques.

Freeze drying

Advantages of this method include long-term survival measured in tens of years, relative stability of characters, ease of storage and freedom from contamination.

Disadvantages are the high capital cost for equipment if commercial freeze driers are used, and the requirement for a considerable amount of skilled technical manipulation.

Freeze drying equipment can be improvised at low cost since the essentials of the apparatus are a vacuum pump, a trap or desiccant to remove moisture and a manifold to connect the apparatus to the (prefrozen) ampoules.

Gelatin discs

In this method, drops of a nutrient gelatin suspension of micro-organisms are dried over a desiccant under vacuum. The resulting dried gelatin discs are stored in screw-capped vials over silica gel.

Advantages of this method include survival measured in years, freedom from contamination, stability of characters, fairly simple manipulation, ease of use and no requirement for expensive equipment.

This method is particularly well suited to storage of frequently-used control strains, as a viable subculture is prepared easily by dropping a disc into warm broth which is then plated and incubated.

An option worth considering is central preparation of gelatin disc-preserved control strains which can be distributed to other laboratories.

Maintenance of bacteria on glass beads

Storing cultures at –70 °C or in the vapour phase of liquid N_2 (–140 °C) is a convenient and reliable method of maintaining a culture collection, but repeated freezing and thawing during subculture damages the bacterial cell.

To overcome this problem, a method using frozen bacterial suspensions with a cryoprotectant in glass beads was developed (Feltham et al., 1978).

A. Materials Required

5% glycerol nutrient broth
Cryotubes (Nunc)
2 mm glass embroidery beads
Sterile loops
Sterile plastic pastettes
Thermal block (kept in the –70 °C freezer).

B. Procedure

I. Preparation of beads

1. Wash the glass beads in tap water with a detergent (e.g. Flow labs 7 x PO_4 free lab. detergent), followed by an acid (0.1 M HCl).
2. After the acid wash, rinse the beads in several volumes of tap water until the pH of the wash water is the same as that of tap water.
3. Give the beads a final rinse in distilled water before placing in the hot air oven to dry.
4. Place 20–30 beads in 2 mL screw-capped cryotubes. Add sufficient glycerol nutrient broth to just cover the surface of the beads. Cap the tubes and autoclave at 15 psi (121 °C) for 15 minutes.
5. Remove the tubes from the autoclave.

The tubes can be stored for six months at room temperature.

II. Growth and harvesting of organisms

1. Grow bacteria on an appropriate non-selective, solid medium under the appropriate growth conditions.
2. Before harvesting the growth, confirm that the culture is pure and that colony morphology is typical.
3. Using a sterile plastic loop, remove growth from the surface of the agar and suspend in the glycerol nutrient broth.
   - Repeat this process several times until a very dense suspension is obtained.
   - Aspirate the suspensions several times to ensure that air bubbles inside the beads are displaced by the bacterial suspension, remove any excess broth and discard into hycolin.
   - Put the top back onto the tube and label with the strain number of the organism. After collecting sufficient growth, use the loop to inoculate a purity plate.
   - Place tubes at –70 °C or in liquid nitrogen vapour.
4. Incubate the purity plate and confirm purity the following day.

III. Recovery

1. It is important to work quickly when organisms are to be retrieved.
   - To minimize loss of viability, the contents of the cryotubes must not be allowed to thaw.
   - Transfer the cryotube from the rack and place in the well of the prechilled (–70 °C) thermal block.
2. Unscrew the cryotube and remove a glass bead with a pair of fine forceps (flame sterilized) or a plastic inoculating loop.
   - Place the bead on the surface of the agar and spread it to form a primary inoculation. Streak the primary inoculation for single colonies, and incubate the medium under appropriate conditions.
3. Immediately return the cryotubes to liquid nitrogen, ensuring that they are placed in the correct location.

Preparation of Simulated Specimens for External Quality Assessment in Microbiology

Design of simulated specimens

A laboratory's performance with quality assessment specimens should reflect its performance in processing patient specimens. Simulated specimens must be realistic in terms of day-to-day general diagnostic bacteriology.

Most of the specimens examined by general diagnostic laboratories either contain no pathogens or yield easily identifiable pathogens.

Bacteriology specimens

To ensure smooth running of the scheme, specimens should be prepared well in advance of the distribution date.

Simulated specimens must be realistic in terms of day-to-day general diagnostic bacteriology. These should be prepared well in advance to ensure smooth running of the scheme.

Steps in the preparation of simulated general bacteriology specimens

1. Retrieval of organisms from storage in liquid nitrogen or at –70 °C.
2. Confirm purity and characterize organisms.
3. Sub-culture organisms to slopes.
4. Harvest cell growth.
5. Dilute, dispense and freeze dry.
6. Perform postfreeze dry QC.
7. Examine the plates and confirm that the specimen has passed post-freeze dry QC checks.
8. Store the organism prior to routine QC.

A. Materials Required

Bacterial strains

Media (relevant to the type of specimen must be used)
Saline 5 mL volumes
Inositol Serum Broth (ISB) 5 mL volumes kept at –30 °C
Gilson pipettes 100 and 1000 mL
Nutrient broth
Agar slopes in 4 oz medical flats
Sterile swabs
Sterile vials
Sterile bungs
Forceps
Sterile plastic loops

B. Procedure

I. Subculture of strains

1. Remove the strains from storage, subculture to plates of appropriate non-selective media and incubate.
2. Confirm purity and characterize the strains to confirm identity.

II. Inoculation of medical flats

1. Choose an appropriate non-inhibitory medium and optimum incubation conditions for organisms.
2. Use a sterile swab to remove growth from the surface of the agar plate and resuspend it in 2 mL of saline. The density of the final suspension must be equivalent to Macfarland standard six (approximately 2.0 x 108 orgs/mL).

The standard must be prepared fresh each week.

3. Use a sterile pasteur pipette to remove 1 mL of the suspension and add a drop to the labelled purity plate; add the remainder to the agar slope. Spread the drop of inoculum on the plate to obtain single colonies. Tighten the cap of the medical flat and tip the bottle several times to inoculate the surface of the agar. Loosen the cap and inoculate the slope and plate under the appropriate conditions.

III. Harvesting of growth

1. Inspect the purity plates for contamination.
2. Use a sterile pasteur pipette to remove the water of condensation from the slope; discard it into a suitable disinfectant.
3. Aseptically add 4 mL of nutrient broth to each agar slope.
4. Use a sterile swab to remove all the growth from the surface of the agar.
5. Homogenize the suspension by aspirating with a pasteur pipette and transfer the suspension to a labelled bijou bottle. Some organisms may not form a homogenous suspension during aspiration (e.g. *Streptococcus* spp.); in such cases, the harvest must be homogenized on a vortex mixer.
6. Dilute the suspensions in nutrient broth to a level shown by experience to produce realistic growth mixtures following freeze drying. The degree of dilution necessary will depend on several factors including the intended level of difficulty of the specimen in terms of the absolute and relative numbers of pathogens and commensals and the loss of viability caused by the freeze drying process.
   Until experience of these factors is gained, trial batches in small volumes using various dilutions of pathogens and commensals can be prepared, freeze dried and examined in order to choose optimal dilutions before large-scale production.
7. Add the broth dilutions to inositol serum broth and mix well.
8. Dispense 0.5 mL of the inositol serum broth into 2 mL vials. Partially insert rubber bungs into the vials, leaving the evacuation channels of the bungs open to allow vapour flow.
9. Freeze dry the vials and when dry, close the bungs completely under vacuum.
10. Immediately after drying, reconstitute the contents of several vials in 1 mL volumes of nutrient broth and subculture to a range of media likely to be used by participants. Ascertain that the absolute and relative numbers of pathogens and commensals are satisfactory and that the colony appearance is typical. Check the consistency of results between vials.

    The vials may now be stored at room temperature or in the refrigerator. If the freeze drying procedure is satisfactory, mixtures should remain viable for several months.

    Several weeks before the planned distribution of vials to participants, the quality of the previously prepared material should be checked. Reconstitute several vials and examine as mentioned above (No. 10). If available, an electronic spark tester may be used to check vacuum in all vials. This is a useful check as loss of vacuum inevitably leads to deterioration of freeze-dried organisms. Label vials and pack them safely with associated report forms and instruction sheets before dispatch to participants.

Preparation of Fixed Smears for AAFB Microscopy

Specimens for the examination for AAFB comprise heat-fixed smears of purulent sputa.

Three categories of slides can be prepared—negative, weak positive and strong positive.

Weak positive is around one organism per five to ten fields, when examined using Ziehl–Neelsen stain at a magnification of 50x. Strong positive is around one organism per field.

The degree of difficulty can also be controlled by altering the amount of background material present, e.g. leucocytes, cellular debris.

Purulent sputa, both positive and negative for AAFB are obtained from laboratories participating in the scheme.

Work pertaining to the preparation of smears must be done in category 3 containment whilst following containment level three safety precautions including use of exhaust protective cabinets. If gamma irradiation facilities are available, sputa can be irradiated following liquefaction to simplify subsequent handling.

Steps for preparing smears for AAFB microscopy

A. Materials required

AAFB positive and negative sputa
Sputasol
Eppendorf pipettes 100 and 200 mL
Fungizone (stock solutions of 1 mg/mL stored in –30 °C freezer)
10 mL pipettes
Lowenstein–Jensen (LJ) slopes
Bijoux bottles
Colour coded glass slides
Blue paper towel
Eppendorf multidispenser
0.5 mL combitips
Plastic loops (10 mL)
Metal slide trays

B. Procedure

I. Preparation of sputa

1. Using a 10 mL pipette add an approximately equal volume of Sputasol, to the sputum and shake vigorously. Digest for 15–25 minutes until the sputum is homogeneous.
2. After digestion, the negative sputa are pooled into one container and mixed thoroughly. Positive sputa are not pooled.
3. Fungizone must be added to the samples to prevent overgrowth with yeast cells. Using an Eppendorf pipette add working strength (1 mg/mL) fungizone at a concentration of 200 mL per 100 mL of sputum. This gives a final fungizone concentration of 2 mg/mL.
4. The sputum and fungizone must be thoroughly mixed and stored at 4 °C.

II. Preparing slides for distribution

1. In order to determine the optimum working dilution, positive material must be diluted with negative sputum. For preliminary screening, four dilutions, 1:3, 1:4, 1:8 and 1:10, are prepared. The dilutions must be prepared and stored in labelled bijoux bottles. The dilutions are made using the ratios listed in the table on the facing page.
2. Prepare four slides of each of the dilutions.

AAFB-negative sputum		AAFB-positive sputum
Dilution	*Volume (mL)*	*Volume (mL)*
1:3	40	20
1:4	60	20
1:8	70	10
1:10	90	10

3. Stain the slides with auramine, and examine using fluorescence microscopy with a 25x objective. After examining by fluorescence, overstain each slide using the Ziehl–Neelsen stain and examine using light microscopy with a 50x objective.
4. Initial reference counts should be obtained by examining and recording the numbers of AAFB present in 10 fields.
5. The overall appearance of the smear, e.g. background debris, presence of white blood cells and evenness of staining must be noted and recorded on the trial mix preparation form.
6. Once an optimum dilution is selected for a particular specimen, the operation can be scaled up to prepare sufficient slides for all participants. Prepare only one specimen at a time to prevent cross-contamination or mix-up of specimens. Always prepare negative slides first.
7. Slides used in preparing smears are colour coded (pink, blue, yellow and green).
8. Place separate sheets of paper towels in the exhaust protective cabinet and lay the colour coded slides marked side up. It is important to check all slides and ensure that they are the correct way up.
9. Using an Eppendorf multidispenser, aliquot 10 mL volumes on to the slide. The drop should be positioned two-thirds of the way from the marked end of the slide.
10. Use a 10 mL loop to smear the drop over an area of approximately 2 cm and place the slide back on to the paper towel.
11. Once the slides are dry, place them in the metal slide trays. Transfer the trays to a hot air oven and fix for one hour at 100 °C. Pack the trays into a large cardboard box. Store the box at room temperature prior to QC and distribution.

III. Quality control of slides

1. The quality of sample slides must be checked using both fluorescence and Ziehl–Neelsen stain.
2. Slides are examined first after staining with auramine and then overstained using Ziehl–Neelsen.
3. The overall appearance and cellular content must be checked and confirmed as positive or negative. The number of bacilli present in selected slides should also be counted to ensure comparability between slides. Counts over 50 fields are obtained using 25x magnification for auramine and 50x for Ziehl–Neelsen.
4. Slides are labelled and checked to ensure that all the labels are put on the correct side of the smear.
5. The slides can now be released for packing.

Hepatitis B serology

Serum specimens containing varying levels of hepatitis B surface antigen (HBsAg) are included in this type of distribution.

The results of the participants can be evaluated for several markers if required, e.g. HBsAg, anti-HBc, anti-HBs, HBeAg, and anti-HBe.

Specimen preparation

Bulk specimens can be produced annually and stored for use during the year. Strongly positive sera whose HBsAg content is documented are diluted in thrombinized negative plasma. Weakly positive sera may be distributed undiluted, if the volume is sufficient.

Specimen selection

Select a set of specimens for preparation in advance, usually sufficient for three distributions. The criteria for selection are that the set should comprise a range of concentrations of HBsAg, including per distribution.

Selection of assays

Bulk sera

The bulk sera are tested for all markers using the most popular kits used by participants (refer to the most recent summary of results for this information). Where there is no clear favourite then cost must be considered and the least expensive option can be selected.

For bulk sera testing the marker kit used should be the least expensive one.

The following tests can be included:

HBsAg ELISA
Anti-HBc ELISA
HBeAg ELISA
Anti-HBe ELISA

All negative sera used for diluting positives must first be screened for anti-HBs. Only those found negative for anti-HBs should be used as diluents.

Selected sera for current distribution

Following storage of bulk sera, selected sera for the current distribution are retested using the most popular HBsAg ELISA test which was previously used for testing the bulk sera.

Perform all tests on well-mixed thawed aliquots according to the manufacturers' instructions. Include the relevant controls. Record ELISA results when all tests are complete. Compare the indices obtained for this ELISA for the bulk specimens and predistribution tests. If there are large discrepancies, discard the specimen.

Dispensing of specimens

Mix thawed specimens thoroughly. Coarse filter through a sterile gauze. Dispense 1 mL of the sera into labelled 2 mL sarstedt vials. Select one aliquot from the beginning, one from the middle and one from the end of each tray and store for future testing in the event of any doubts about the reproducibility between aliquots. Use the last aliquot from each specimen to perform a sterility check.

HIV Serology

Serum specimens containing various levels of antibody to HIV are included in this distribution. Bulk specimens can be produced annually and stored for use during the year.

Material which is positive for anti-HIV, anti-HCV or HBsAg must be processed in a containment level 2 laboratory. Latex gloves must be worn in addition to normal protective clothing. In order to contain any spillage, all manipulations of plasma packs containing positive material must be carried out in a class 1 safety cabinet.

Spillages of more than a few millilitres must be reported immediately to the safety officer, and all cleaning must be carried out according to local safety regulations.

Anti-HIV positive material should

be heat-inactivated at 56 °C for 30 minutes before handling. Ensure that sera reach 56 °C before starting timing, by placing a thermometer in a bottle containing the same volume of water as the sera, at the same starting temperature as the sera. Do not attempt this procedure with sera taken straight from the refrigerator; allow them to reach room temperature first. Note that heat inactivation does not render the sera free from risk of infection, it only lessens the infectious load.

Specimen selection

In order to conserve precious material, anti-HIV positive sera are diluted. Experience has shown that highly diluted specimens are not suitable for all types of assay, and the dilution factor should not normally exceed 1:50.

The set of sera distributed should contain a range of concentrations of anti-HIV, and a variety of Western blot patterns. Serum negative for anti-HIV should also be included and occasional sera containing anti-HIV 2, or a mixture of anti-HIV 1 and anti-HIV 2 should be used.

Selection of assays

Bulk sera

The bulk sera should be tested for all markers using the most popular kits used by participants. Where there is no clear favourite then cost must be considered and the least expensive

Cleaning of spillages must be carried out according to local safety regulations. Spillages of more than a few millilitres must be reported immediately to the safety officer.

option should be selected. The kits included should represent a range of test methods and antigen sources, for example:

- Gelatin particle agglutination
- Competitive ELISA
- Sandwich ELISA
- Western blot
- Line assay
- Anti-HIV 1 ELISA
- Anti-HIV 1+2 ELISA

Selected sera for current distribution

Following storage of bulk sera, selected sera for the current distribution are retested using the most popular anti-HIV 1+2 ELISA test which was previously used for testing the bulk sera.

Predistribution testing

Perform all tests on well-mixed thawed aliquots according to the manufacturers' instructions. Include the relevant controls.

For anti-HIV 1 positive samples, select the samples with antibodies to several gene products including at least 2 envelope proteins (gp 41, gp 120, gp 160) and p24 in the HIV1 Western blot test (WHO criteria). Line assays should be unequivocally positive for HIV 1 or HIV 2 unless a mixed infection is suspected.

Negative specimens should show no non-specific reactions in any assay, except in the case of a known or suspected false-positive reaction, however, 1 or 2 bands in the Western blot are acceptable if they give an indeterminate pattern.

Sera for current distribution

Remove the required sera from frozen storage and thaw overnight at room temperature.

Mix well and perform the most popular HIV 1+2 ELISA according to the manufacturer's instructions including the relevant controls.

Compare the indices obtained for this ELISA for the bulk specimens and predistribution tests. If there is a marked difference between the two sets of results, investigate.

Dispensing specimens

Mix thawed specimens thoroughly. Coarse filter through a sterile gauze. Dispense 1 mL volumes of the sera into labelled 2 mL vials. Select one aliquot from the beginning, one from the middle and one from the end of each tray and store for future testing

in the event of any doubt about the reproducibility between aliquots. Use the last aliquot from each specimen to perform a sterility check.

Syphilis serology

Selection and preparation of specimens

Considerable diversity in results between laboratories is common when testing for markers of syphilitic infection. The detection of residual antibody often depends on the sensitivity of the kits used. It is difficult to evaluate the EQA results of individual participants unless a reasonable consensus is obtained. For this reason, it is advisable to get sera tested by a number of reference laboratories and use it for preparation of EQA samples only if there is reasonable consensus of results.

Sera may be stored in bulk in the freezer after testing by the reference laboratories.

1. For safety reasons, sera should be tested for anti-HIV, HBsAg and anti-HCV and should be used only if negative for these markers.
2. Select six sera with a good consensus of results so that a range of values in different tests is obtained, e.g. strong/weak/negative VDRL, strong/weak TPHA, strong/weak FTA-Abs.
3. Ensure that the volume of serum is sufficient (0.4–0.5 mL per participant).
4. After thawing, ensure that the serum is thoroughly mixed without coming in contact with the inside of the bottle top. Ensure that the macroscopic appearance of the serum is good. If clots or debris are visible, filter through sterile gauze.
5. Perform sterility tests. If any serum proves to be bacterially contaminated, filter to sterilize and add Bronidox.
6. Store at 4 °C until predistribution testing is complete.

Selection of assays for predistribution testing

Refer to a recent distribution summary and, if required, order the most popular commercial kits for reaginic antibodies to VDRL, rapid plasma reagin test and TPHA.

Performance of assays

- **VDRL**: Perform the test following the manufacturer's instructions. Obtain quantitative results for all positive specimens. Include a known positive control with each batch of tests. Record kit details and results.
- **TPHA**: Perform the quantitative test following the manufacturer's instructions, using automatic pipettes for dispensing and diluting. Titrate the specimens, across the longer side of the plate, up to 1:1280 dilution. Include a known positive control with each batch of tests. Record kit details and results.
- **FTA-Abs**: Perform the test according to the manufacturer's instructions. Include the recommended controls with each batch of tests. Record kit details and results.

When all predistribution tests have been performed satisfactorily, proceed to dispensing the specimens into aliquots.

Thrombin Treatment of Plasma Packs

Safety information

Material which is positive for anti-HIV, anti-HCV or HBsAg must be processed in a class I safety cabinet to contain spillage. Latex gloves must be worn in addition to normal protective clothing.

Spillages of more than a few millilitres must be reported immediately to the safety officer, and all cleaning must be carried out according to local safety regulations.

The QA serum specimens supplied as plasma need to be converted to serum by thrombinization. Although plasma is suitable for testing by most commercial

immunoassays, serum is easier to handle in bulk as it is less prone to spontaneous clotting. Serum is required where specimens are heated, e.g. anti-HIV positive specimens, as plasma would clot on heating.

Plasma is usually derived from blood donations which have been collected in packs containing an anticoagulant; the red cells are subsequently removed by centrifugation. As the natural clotting mechanism is bypassed, in order to obtain serum it is necessary to add thrombin which catalyses the formation of the fibrin clot from the precursor fibrinogen.

Two types of thrombin, human and bovine are available, and while both appear to give satisfactory results, the bovine preparation is preferable on grounds of cost.

Faecal parasitology

Selection of samples for faecal parasitology distributions

Samples are selected from patients with known infections for their suitability for the following reasons:

- Number of species present.
- Intensity of infection.
- Whether characteristics and morphological features of species are shown.
- Availability of sufficient quantities of specimen.

Informed consent of the patient is required and permission of clinicians should be obtained.

To avoid exposing EQA staff and participants to unnecessary risk, material from HIV-negative patients should be used. HIV testing is required. Specialist institutes may be able to help obtain suitable material and use of cultured parasites is sometimes an option.

Preparation of faecal samples for storage

Preparation of positive stool samples

1. Screen samples extensively for suitability using the Ridley and Allen formol–ether concentrate method or the formalin ethylacetate method.
2. Select samples which have moderate to high numbers of parasites, as numbers will be diluted on addition of formalin.
3. Record all the parasites present. There must be no more than three parasites present in one sample, although if small numbers of additional parasites are present which are not in sufficient numbers for scoring, the sample can still be useful but the parasite will not be included in the scoring of the specimen.
4. Add 10% formalin to screened specimens to preserve parasites and either process immediately or refrigerate until required. Cysts tend to lose their morphology on prolonged refrigeration i.e. >1 year. Ova, however, can be stored indefinitely.
5. Leave in 10% formalin overnight before processing the high-risk specimens (i.e. patients with HIV or hepatitis).
6. Label sample containers clearly and quantify parasites as +, ++, +++, etc. Also code the sample container with numbers (i.e. F1, F2, etc.) and record parasite content in a specimen book.

Preparation of negative stool samples

Occasionally, stool samples with no parasites are collected for the following reasons:

- There may be used as part of distribution as a negative specimen.
- Ova or cysts supplied by specialist institutes or cultured parasites can be added to negative stools.

Negative stools are obtained from

- People with no signs or symptoms of parasitic disease.
- People with no recent history of foreign travel.

Negative stools must be screened extensively to exclude all parasites. This is done by using the Ridley and Allen formol–ether concentration method and making faecal smears.

Preparation of 1000 units/mL bovine thrombin solution

A. Materials required

Bovine thrombin; Sigma Chemicals, Cat. no. T4648

Sterile distilled water with 0.1% bovine serum albumin

Plastic vials ('Sarstedts')

Plastic pipettes.

B. Procedure

1. Wear gloves and work in a Class 1 safety cabinet. Each vial contains approximately 10,000 NIH (National Institutes of Health) units thrombin. Aseptically reconstitute the contents of the vial with 10 mL distilled water with 0.1% BSA.
2. If the whole volume is not to be used immediately, aliquot the solution in 1 mL volumes in plastic vials and label with contents, batch, date, volume and number of units/mL. Store frozen below –30 °C.

Use the solution at the rate of 1 unit of thrombin per 1 mL of plasma, i.e. 100 mL (100 units) per 100 mL plasma.

Thrombinization of plasma

A. Materials required

Bovine or human thrombin solution

8" x 6" polythene bags

Plastic washing-up bowl(s)

Scissors (2 pairs)

Autoclave bin (lined with an autoclave bag), and lid.

Disposafe jar

70% alcohol

Presept (chlorine-releasing) granules

500 mL glass bottles

250 mL square bottles

Sterile plastic vials ('Sarstedts')

Disposable plastic pipettes

Beaker containing 70% alcohol

B. Procedure

1. Assemble all materials required and remove appropriate plasma packs from the freezer.
2. Label and record details.
3. Place each pack individually into a strong plastic bag and place upright in a plastic bowl. Leave the packs

to thaw overnight at room temperature on the bench.

4. Place a lined autoclave bin on a trolley next to the cabinet and within easy reach.
5. Using a plastic tape, label one 500 mL Schott bottle per pack with the QAL and full pack numbers.
6. Transfer to bottles using a transfer tube.
7. Swab the working area with 70% alcohol.
8. Estimate the volume of each plasma pack from the gradations on the bottle, to the nearest 50 mL. Add the appropriate volume of thrombin to each plasma pack. Mix very gently, ensuring that the plasma does not come into contact with the lid of the bottle. Place in an incubator at 37 °C for 1–2 hours.
9. After incubation, check that the plasma has clotted. Some may require further incubation.
10. Remove bottles from the incubator. Allow plasma to cool for one hour at room temperature and then freeze at –30 °C. Best results in terms of clarity of product are obtained by leaving the serum for 2–3 days at –30 °C before thawing and removing the serum from the clot.
11. To thaw, place the bottles in a plastic bowl and leave for several hours. Label 250 mL square plastic Nalgene bottles or glass Schott bottles with plastic tape bearing the code and pack numbers, date of thrombinization and with any appropriate hazard warning.
12. Holding the clot back with a pipette pour the serum into the bottles,. Extract the maximum volume from the clot by pressing with the pipette, leaving the pipette in the bottle and repeating at intervals as the clot retracts, over a period of approximately one hour.
13. Take samples for predistribution testing as follows: for each pack take 3 x 2 mL plastic vials and label them appropriately. Pipette 1 mL of serum into each vial and store it with the bulk specimen, at 4 °C if processing for immediate use is to continue, or at –30 °C until required. If glass bottles are used, these must be sealed in plastic bags before freezing, in case they crack on thawing. Prolonged storage (i.e. for more than 3 years) is not desirable, as it can affect the physical quality of the specimen.
14. Before use, test for sterility using one of the reserved samples.

Preparation of faecal samples for distribution

Select a screened sample from the refrigerator and add it to an industrial blender and homogenize until semiliquid. It may be necessary to add 10% formalin in order to:

- Achieve the consistency required for dispensing.
- Dilute the number of parasites per mL if the parasite concentration is too heavy or if parasites are being added to a negative stool.
- To attain sufficient amount for distribution (approximately 600 mL).

The parasite numbers are further checked before deciding if the specimen is ready for bottling.

The process of diluting, homogenizing and checking parasite numbers should be repeated until the specimen is in the required state for bottling. Approximately 550 two mL serum tubes are required for 520 participants. Repeat specimens and return samples are arranged in metal trays and 1 mL of faecal sample is added to each vial with constant stirring. This method has proved to be the most useful as there is a lot of sample wastage if an electrical dispenser is used.

Specimen trays are clearly labelled and stored in the refrigerator until

required. Samples containing helminth ova may be kept for several years. Samples containing cysts, however, should be used within a few months.

Preparations of samples other than faeces for distribution

a. Terminal urine for ova of *Schistosoma haematobium*

- Urine should be collected and screened for suitability of use as in the case of faecal samples.
- Add 10% formalin to the urine to preserve the ova and refrigerate until ready for use. The final volume should be approximately 600 mL.
- Approximately 550 two mL serum tubes are required for 520 participants. Repeat specimens and return samples are arranged in metal trays and 1 mL of urine sample is added to each vial with constant stirring.
- Specimen trays are clearly labelled and stored in the refrigerator until required.
- Ova of *Schistosoma haematobium* tend to become granular on prolonged storage, therefore it is advisable to dispatch them within 6 months of receiving them.

b. Hydatid cyst fluid for protoscolices of *Echinococcus granulosus*

- Cyst fluid should be collected and screened (as in faecal samples) for suitability.
- Add 10% formalin to the cyst fluid to preserve the protoscolices and refrigerate until ready for use.
- The final volume should be approximately 600 mL.
- Approximately 550 two mL serum tubes are required for 520 participants. Repeat specimens and return samples are arranged in metal trays and 1 mL of cyst fluid is added to each vial with constant stirring. This method has proved to be the most useful as there is a lot of sample wastage if an electrical dispenser is used.
- Specimen trays are clearly labelled and stored in the refrigerator until required.

Faecal smears

Faecal smears may be sent as part of a distribution to examine for oocysts of *Coccidia* or trophozoites of certain protozoa. The faecal sample should first be screened for suitability. If *Coccidia* oocysts are present, the faecal sample is fixed in 10% formalin to preserve the oocysts.

If trophozoites of *Giardia lamblia*, *Dientamoeba fragilis*, *Blastocystis hominis* or other flagellates are present, the slides must be made immediately. 550 thin faecal smears are made on glass microscope slides and fixed in methanol. The fixed smears are stored in the slide cupboard if oocysts are present. All other smears must be stained immediately.

Smears can be sent out either stained or unstained depending on diagnostic requirements, as well as on the particular parasite to be examined.

Simulated specimens

Cultured parasites, namely trophozoites of *Giardia lamblia* and filariform larvae of *Strongyloides stercoralis* may be sent out as part of a faecal parasitology distribution. Both parasites can be:

- Added to a negative faecal sample.
- Used as a simulated specimen of sputum to examine for filariform larvae of *Strongyloides stercoralis* or jejunal aspirate to examine for trophozoites of *Giardia lamblia* and filariform larvae of *Strongyloides stercoralis*.

Preparation of simulated specimens

Sputum

Simulated sputum is prepared by scraping the inside of the cheek and adding the cells to 10% formalin. Larvae of *Strongyloides stercoralis* can then be added to this preparation.

Jejunal aspirate

Simulated jejunal aspirates can be prepared using the medium for culturing trophozoites of *Giardia*

lamblia. This medium is yellow and resembles jejunal aspirate. Cells from the inside of the cheek can then be added, thus making the sample more authentic.

Guidelines for suitable parasite numbers for distribution

All laboratories must find the parasites that are present by using the relevant diagnostic technique although sample variations are possible. The following guidelines should be adhered to while using the Ridley and Allen formol–ether concentration method:

- A faecal deposit should contain more than five ova per deposit.
- A faecal deposit should contain more than one cyst per 20 high power fields (40x objective).
- When direct examination is required as in urine, cyst fluid or simulated specimens a coverslip should contain more than one ovum, larva or protoscolex or more than one trophozoite per 20 high power fields (40x objective).

Quality control of bottled samples prior to distribution

Select 10 samples at random for every 500 samples bottled.

For faecal samples, use the Ridley and Allen formol–ether concentrate method to concentrate the available samples. For non-faecal samples, examine the sample by direct microscopy. For faecal samples examine all the deposit and record the numbers of all parasites present as follows:

- Count ova and larvae as number per deposit in a faecal sample.
- Count the number of cysts as an estimate of the number of parasites per high power field (40x objective) as it is impractical to count the number of cysts per deposit.

For non-faecal samples, examine and record the number of parasites present as follows:

- Count the number of ova, larvae, trophozoites or protoscolices per coverslip.

Faecal smears may contain scanty, moderate or numerous parasites per smear

Numerous	1–>5 parasites per oil immersion field (100x objective).
Moderate	Approximately 5 parasites per 10 oil immersion fields (100x objective)
Scanty	Approximately 1 per 10 oil immersion fields (100x objective).

- Calculate the range and average number of parasites.
- The stage of parasite is noted.
- Comment on the specimen.
- Note any extraneous parasites.

The quality control information should be recorded on a form and kept in a folder marked 'specimen preparation'. A copy should also be included in the appropriate distribution details file once distribution number and specimen numbers are known. Give a coded sample to all the staff in the clinical laboratory and ask them to record their findings on a form. Only once the sample has been thoroughly screened can it be considered suitable for distribution.

Quality control of samples after distribution

To check the effects of transit and possible changes of the specimen once opened, five laboratories should return a duplicate set of specimens unopened.

The five selected laboratories are the same for every distribution. Once these samples arrive, check the following:

- The quality of the parasite is the same as recorded.
- The number of parasites present is the same as that recorded.
- Re-examine for any extraneous parasites.
- Record the information on a form.

If the sample has been severely altered in transit, it is not scored and is recorded in the non-compliance SOP. Once these samples have been found to be satisfactory, the preparation of the summary of participants' reports can begin.

Blood Parasitology

Selection of samples for blood parasitology distributions

The selection of samples is based on the following factors:

- Number of parasites present in a stained thin blood film.
- Stages of parasite present.
- Whether characteristic morphological features of species are shown.
- Availability of sufficient quantities of the specimen to make the required number of blood films.

Preparation of blood samples

Preparation of positive blood samples

Slides should be made immediately after infection is diagnosed.

Do not use specimens when

- Samples have been left overnight, as parasite morphology will be altered.
- Patient has been partially treated, as parasite morphology will be altered.

Screen material extensively, using thick and thin blood films and stain with the appropriate staining method.

A sample can be suitable for distribution only after it is thoroughly screened.

Record the number and stages of parasites present and ensure that they are in sufficient numbers for them to be present in every film. Ensure that the morphology is characteristic of the given parasite as advised by senior laboratory staff. Only when the suitability of a specimen is ensured can the specimen be processed and stored.

Preparation of negative blood samples

Blood samples with no parasites are collected for the following reasons:

- They may be used as part of a distribution, as a 'negative' specimen.
- Cultured parasites can be added to negative blood.

Negative blood is obtained from:

- People with no signs or symptoms of parasitic disease.
- People who have had no exposure to tropical disease (i.e. have not visited a tropical country).

Negative blood must be screened extensively to exclude all parasites. This is done by making thick and thin blood films and staining with Giemsa stain.

Preparation of thin blood films from blood samples for distribution

Once the blood sample is selected, films are made for distribution. Only good quality microscope slides are used for distribution because dirty or greasy slides will effect the morphology of parasites. Substandard slides are not used for the preparation of blood films.

The number of slides made is based on the number of participants, which is continually revised. Gloves must be worn at all times. Once blood films are made they are put into slide racks, fixed in methanol and stained with the appropriate stain at the appropriate pH.

Blood films may also be fixed in methanol and stored in the -20 °C freezer for up to 6 months prior to staining. This is done only if there is insufficient time to stain the films.

Fixed blood films must not be stored at room temperature for over 2 days as the blood may get oxidized. This results in excess stain deposit and also affects the staining of the parasites. Only stained blood films can be stored at room temperature.

The dry stained blood films are then removed from the slide racks, put into slide boxes and stored at room temperature until required.

Slide boxes are carefully labelled with the parasite, stage(s) present, parasitaemia where appropriate and the date slides are made. Also code the box with a storage number and record it in the specimen book.

Blood films may be stored indefinitely once they are fixed and stained.

High-risk specimens

If the specimen is of high risk it is labelled 'Danger of Infection'. This indicates that the specimen potentially or actually contains HIV or Hepatitis viruses and that the following precautions must be taken.

- The slides must only be prepared by experienced staff.
- Gloves must be worn at all times.
- The films are made in a Class 2 safety cabinet.
- The films are fixed for 15 minutes in methanol.
- The films are then fixed for 15 minutes in ethanol.

Appropriate staining can then be carried out.

Preparation of samples other than thin blood parasitology distributions

Thick blood films for all blood parasites

Make thick blood films. Stain and fix films with acetone post staining.

The films are then screened as follows:

- Record all the stages of malaria parasites present.
- Record the parasitaemia if parasite is *Plasmodium falciparum*.
- Count the number of microfilaria present.

Bone marrow for amastigotes of *Leishmania donovani*

Bone marrow should be collected and thin films made. If the patient is infected with HIV, follow the precautions outlined above. Screen films for the presence of amastigotes of *Leishmania* species.

Tissue dabs for amastigotes of *Leishmania donovani*

Usually splenic dabs (from mouse spleen) are made to examine for amastigotes of *Leishmania donovani*.

Slides are made by dabbing a piece of tissue on microscope slides followed by fixing and staining with the appropriate stain.

Films are screened for the presence of amastigotes of *Leishmania* species.

Cultured blood parasites

Clinical samples containing trypomastigotes of *Trypanosoma brucei* species and *Trypanosoma cruzi* are scarce and parasites cultured in mice can be used in a distribution either:

- Washed and added to a negative human blood sample. The samples should be processed using the procedure in the section on the preparation of thin blood films, fixed and stained and screened for suitability of parasites.
- Processed as thick films using mouse blood. Samples should be processed as in section concerning the preparation of thick blood films and screened as in SOP concerning quality control of blood films.

Care must be taken when dealing with *T. cruzi* as it is a category 3 pathogen. *Leishmania donovani* sp. can also be passaged in mice and the spleen supplied.

Use only
good quality slides as dirty and greasy slides effect the morphology of parasites in the blood film.

Quality control of stained blood films

Select five samples for every 500 slides.

Examine the whole slide and record the parasites present on a form as follows:

- Record all the stages of malaria parasites present.
- Record the parasitaemia, if parasite is *Plasmodium falciparum*.
- Count the numbers of microfilaria present.

The quality control information should be recorded and kept in a folder marked 'specimen preparation'. A copy should also be included in the clinical laboratory to record their findings as check of the specimens' suitability for distribution.

Give a coded sample to all the staff in the clinical laboratory to record their findings as a check of the specimen's suitability for distribution. Only once the sample is thoroughly checked can it be considered suitable for distribution.

Quality control of samples after distribution

To check the effects of transit and possible changes in the specimen once opened, five laboratories should return a duplicate set of specimens unopened. These five selected laboratories are the same for every distribution.

Once these samples arrive, check the following:

- The identity of the parasite is the same as recorded.
- The quality of the parasite is the same as recorded.
- The number of parasites present is the same as recorded.
- The stages of the parasite are the same as recorded.
- The staining of the parasite is satisfactory.

Re-examine for any stages which may be present in small numbers and may have been missed during the quality control of the specimen. Record the information on the form for checking returned specimens. If the specimen has been severely altered in transit, it is not scored and recorded in the non-compliance SOP.

Once these samples have been found to be satisfactory, the preparation of the summary of participants' reports can begin.

6 External Quality Assessment in Serology of Bloodborne Infectious Agents

IT IS NOW mandatory in many countries to test all blood donations for bloodborne infections.

It would be preferable to conduct the EQAS in the serology of bloodborne infections in collaboration with an organizer conducting EQAS in viral serology.

Test exercises

1. **HIV antibody**
2. **HBs antigen and HBe antibodies**
3. **HCV antibodies**
4. **VDRL.**

Materials Required

Serum containing antibodies collected from patients, titrated and filtered. (See preparation of analytes later.)

Number of exercises in a year: Four, concurrently with Blood Bank serology exercises.

Dispatch of test materials: By post to nearby areas. By air to distant areas. The materials should arrive at their destination within two weeks.

Testing and Report by participants: EQA materials should be tested in the normal way, as other specimens are tested. Within two weeks, the report should be sent to that organizing laboratory.

Evaluation of participants: The organizer should evaluate the results sent by participants and send them his report within two weeks. The organizer should also prepare an annual report on the performance of all participants, reporting only the trends of the participating laboratories and maintaining strict confidentiality.

The EQAS of serology of bloodborne infections should be conducted along with the organizers of EQAs in viral serology.

For poor performers: As in all the EQASs, training must form an integral part of the NEQAS and the training programme should be prepared by the Advisory Committee of which the organizer is also a member.

The Advisory Committee should meet at least once an year to evaluate the scheme, decide further programmes and also liaise with the accreditation authorities.

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Chapter 4

Quality Assurance in Haematology

1. Introduction

HAEMATOLOGICAL TESTS are largely based on enumeration of various types of cells and qualitative analysis of cell characteristics. Other tests include quantitation of plasma, concentration of coagulation factors and quantitative analysis of serum for certain constituents. The basis of diagnostic haematology is the blood count which comprises the bulk of work in a routine haematology laboratory. Special attention will, therefore, be given to this aspect in this section.

A problem in standardization is that few reference standards are available. Interlaboratory comparisons by means of external quality assessment schemes (EQASs) provide a means for laboratories to achieve an appropriate degree of harmonization. However, there is no guarantee that the mean, median or mode of results from the group of laboratories participating in the EQAS will represent the true answer for any test parameter.

Accuracy can only be achieved by using standards and calibrators

It must also be recognized that it is likely that in this way laboratories may become harmonized to the same incorrect value. Accuracy can only be achieved by using standards and calibrators. Calibrators and controls may be identical preparations, but their difference lies in the fact that values are assigned to calibrators by reference methods, linked to primary standards if possible, whereas for a control the important specification is the optimal standard deviation when it is tested along with batches of specimens.

Internal quality control (IQC) is fundamental for reliable haematology. Participation in an appropriate EQAS is also an important component of quality assurance (QA). IQC and EQA complement each other and are not alternatives.

2. Analytic Tests

HAEMATOLOGICAL TESTS that should be available at the different levels of the laboratory are given below:

Peripheral Laboratory

- Haemoglobin
- ESR
- Stained blood film
- DLC
- Red cell morphology
- Platelet count (chamber or film)
- Tests for detection of malaria.

Intermediate Laboratory

In addition to the tests mentioned above, the intermediate laboratory should have the facilities to conduct the following:

- PCV
- RBC, WBC and platelet count (electronic counter) MCH, MCV, MCHC
- Reticulocytes
- Sickle cell test
- Antihuman globulin (Coombs') test
- Prothrombin time
- INR
- G6PD screening
- Complicated blood film morphology (e.g. leukaemias)

Central Laboratory

In addition to the tests mentioned above, the central laboratory should have facilities for:

- Hb electophoresis
- Hb A2
- Hb F
- APTT
- Fibrinogen
- Coagulation factors
- Anticoagulant dose assessment
- Serum B12, folate, ferritin, iron.

3 Control Materials

MATERIAL FOR both IQC and EQA should, as far as possible, resemble the patients' specimens in the analytic process. Control material should be sterile and stable for a reasonably long time (at least three to four weeks).

When a preparation is distributed into vials, the aliquots must be homogeneous, especially for cell counts. In practice, material which has been stabilized by fixation does not always behave exactly like patients' specimens, and this must be recognized. Preserved blood behaves more like fresh blood, but is stable for a shorter time than fixed preparations. Above all, the material must be readily available. Many laboratories, either individually or collectively, prepare their own material. However, even when it is necessary to purchase material from commercial sources, laboratory staff must appreciate the importance of having functionally adequate control material and regard it as a necessary and valued reagent and not merely a desirable cost-free adjunct.

Control material is a valued reagent, not just a cost-free adjunct. Control material should be sterile and stable for a long time.

Material for Blood Counts

The following material may be used as calibrators and control preparations for blood counts. Their preparation is described in the section on Preparation of Control Materials.

A. Haemoglobin (haemiglobincyanide, HiCN) reference preparations.
B. Lysate of haemolysed red cells.
C. Partially fixed stabilized blood.
D. Blood preserved in acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD).
E. Fixed avian (e.g. chicken) red cells, for use as pseudo-leucocytes, suspended in leucocyte-free preserved blood.

F. Fixed human platelets; difficult to prepare, but batches are made at the WHO Collaborating Centre for use in the International EQAS.

Calibrators

The haemoglobin concentration of preparation A should be determined by measuring its absorbance at a wavelength of 540 nm in a spectrophotometer alongside a sample of the international haemoglobin standard as described in the WHO document LAB 84.10[4]. This preparation can then be used for calibrating haemoglobinometers (see WHO/CDC Manual—Fundamental diagnostic haematology: Anaemia[2]).

For blood count parameters, values may be assigned to preparations B, C, D or E by the International Council for Standardization in Haematology (ICSH) recommended reference methods as described in S.M. Lewis, Quality Assurance in Haematology[1].

Internal Quality Control of Blood Counts

For IQC of blood counts, laboratories should have preparations B, C, D and/or E. Aliquots of 3–5 mL should be dispensed in vials/tubes, which must be tightly stoppered and kept in the refrigerator. Fresh samples must be used on each occasion.

It is important to ensure that the aliquots are homogeneous when dispensed, and that they are discarded if they become contaminated (a fresh batch should then be prepared). The methods for making these preparations are described in the section on Preparation of Control Materials.

In the first instance, 15–20 tests are carried out on 2–3 samples from the batch using the routine methods of the laboratory as carefully as possible. The mean and standard deviation are obtained. Subsequently, daily (or on each batch of tests in the day) a control sample is analysed alongside the patients' specimens. Results are plotted on a control chart with the value obtained on the *y* axis and the day (or batch) on the *x* axis. The position of these plots is noted in relation to the established 1, 2 and 3 SD. Their significance is interpreted as described in Chapter 1.

4 Other Internal Quality Control Procedures for Blood Count

WHILE CONDUCTING TESTS with control preparations and charting the results each day is the most important IQC procedure in the laboratory, there are other procedures which should be carried out as part of total QA.

Correlation Check

Unexpected results must be checked to see whether they can be explained on clinical grounds or whether they correlate with other tests. For example, an unexpectedly higher or lower haemoglobin level might be explained by a blood transfusion or a haemorrhage, respectively.

A low mean corpuscular haemoglobin concentration (MCHC) should be confirmed by demonstrating hypochromic red cells on a Romanowsky-stained blood film; a high mean corpuscular volume (MCV) must correlate with macrocytosis; similarly, the blood film should be examined to confirm a leucocytosis or leucopenia, thrombocytosis or thrombocytopenia—but with care, as the blood film itself may be misleading if not correctly made and stained.

Recording blood count data on cumulative report forms is a good

clinical practice. It provides an in-built QC system by making it easy to detect an aberrant result when compared with a previously determined baseline. This is especially useful in detecting occasional wild errors caused by incorrect labelling, inadequate mixing or partial clotting of a blood sample.

A formal way of testing aberrant results is known as 'Delta check'. The blood count parameters should not differ in subsequent tests by more than a certain amount, taking into account both test coefficient variance (CV) and physiological variation.

In laboratories with automation, the differences should generally not be more than 10% for Hb and RBC, 20% or a change between normal and abnormal for WBC, and 50% for platelets, assuming that the patient's clinical condition has not altered significantly.

Duplicate Tests on Patients' Specimens

This is the easiest procedure to carry out. If the day's work allows it, every specimen should be tested in duplicate; and if there are too many test requests for this to be practical, at least four to five consecutive specimens should be tested in duplicate from time to time.

Calculate the standard deviation:

Recording blood count data on cumulative report forms provides an in-built QA system and helps in determining aberrant results.

$$SD = \frac{\sqrt{\Sigma \text{ of } d^2}}{2n}$$

d = difference between duplicates

n = number of pairs

None of the duplicate tests should differ from each other by more than 2 SD.

Patient Data

In a large hospital where at least 100 blood counts are performed each day, there should be no significant day-to-day or week-to-week variability in the mean of red cell indices (MCV, MCH, MCHC). This is a useful method for QC in laboratories with automated cell counters, as any significant change may indicate a change in instrument calibration or a fault in its function, provided that the specimens are evenly distributed.

The more sophisticated automated cell counting systems have a computer program incorporated in the system which makes it possible to analyse the data continuously. With other counters, results can also be analysed using a programmable calculator or personal computer. By this means, results are analysed in successive batches of 20 specimens.

Results are valid only if the population from whom the routine specimens are received does not vary significantly from day-to-day and the tests are not selectively biased. Such a bias might occur if, for example, as a result of specific outpatient clinics, tests are carried out only on certain days of the week on patients with iron deficiency or with some other condition which affects the MCHC, MCH and/or MCV. To overcome this problem, results from these clinics should be excluded.

A simple adaptation of the same principle can be applied in laboratories which do not have computer facilities by using manual methods. In this situation, the procedure is confined to the MCHC. The mean MCHC is calculated at the end of each day on all the measurements obtained during the course of the day.

If the test is being performed satisfactorily, the mean will not vary by more that 2 SD on any day. Before setting up this procedure, it is necessary to establish the SD. This is done by calculating the mean on 11 consecutive working days and then calculating the SD. Thereafter, any significant alteration in the daily

mean indicates that a fault has occurred. It is convenient and educative to plot the data on a graph. The component indices (Hb and PCV) are controlled only indirectly by this procedure.

The mean MCHC is calculated at the end of each day on all the measurements obtained during the course of the day.

Other Tests

The best method for IQC for tests for which controls are not available is to carry out the tests in duplicate and to follow the recommendations in the WHO/CDC manuals [2,3].

5. External Quality Assessment in Haematology

Blood Counts

Preparations A and B are suitable for haemoglobinometry, while preparations C, D and E are suitable for blood cell counts and related parameters. The instability of preserved blood makes it less suitable than partially fixed blood when participants are unable to receive specimens within seven to ten days. Partially fixed blood can be used for about three to four weeks. Preparation E is recommended for WBCs as it is stable for several months.

Other External Quality Assessment Preparations

Blood films

Suitable preparations include stained films from normal subjects and patients with various diseases. Participants are required to identify abnormal cells and perform differential leucocyte counts. Films with malaria or other blood parasites should also be included either as an unknown' or identified as a 'blood parasite survey'; the latter is intended as a test for classification and quantification of parasitaemia. In some surveys, the films are fixed but not stained, so as to allow participants to carry out the staining themselves. This is successful only with relatively fresh films which have been fixed immediately after preparation.

Reticulocytes

Suitable preparations for reticulocytes include blood films which have been pre-stained with New Methylene blue or Azure B.

Haemoglobin electrophoresis and Hb A2

Lysates from appropriate subjects are suitable preparations for control tests.

G6PD

Blood in acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD) (preparation D) from a normal subject is suitable. Sheep blood is also useful as it has reduced levels of G6PD.

B12, folate, ferritin, iron

Sterile serum is suitable for use in EQA.

Coombs' test

IgG and complement-sensitized red cells in ACD or CPD are suitable for use in EQA.

Prothrombin time and other coagulation tests

Lyophilized plasma may be used and reconstituted in the laboratory immediately before testing.

Preserved blood is less suitable than partially fixed blood because of its instability.

International External Quality Assessment Schemes

The World Health Organization sponsors a project in which selected laboratories in different countries take part in an international EQAS (IEQAS). This is organized by the WHO Collaborating Centre for Quality Assessment in Haematology in association with the United Kingdom national scheme (NEQAS).

Postal and customs delays often occur under unfavourable climatic conditions. Thus, only stable materials can be sent to participants worldwide. These include lysates for haemoglobin; stabilized blood for RBC, WBC and platelet counts; Romanowsky-stained blood films for DLCs, morphological diagnosis and identification of blood parasites; and sterile serum for vitamin B12, folate, iron and ferritin.

Laboratories in the WHO/EQA scheme should subsequently be responsible for organizing regional and national schemes.

The material used in IEQAS surveys consists of either specially prepared stabilized blood or the same preparations as distributed in the UK scheme. The preparations are measured by experts in the Collaborating Centre using reference methods. Limits are established for satisfactory performance as described above. International participants also receive a report containing the results of the UK surveys, and as the statistical evaluation in the UK NEQAS is based on over 800 results obtained by various techniques, including different types of automated cell counters, the consensus is unlikely to be affected by bias.

The SD and CV are remarkably low in the UK scheme and this sets the standard for international participants who can thus judge their performance critically, although their own results will not have been included in the statistical evaluations.

The intention is that laboratories in the WHO scheme may subsequently be responsible for organizing regional or national schemes which will thus be linked at an international level. Further information is obtainable from the Laboratory Unit (LAB) of the World Health Organization, Geneva, or from the WHO Regional Offices.

6 Scoring System for Assessing Performance in an External Quality Assessment Scheme

Target Values

A target value is the value which is used for assessing the performance of participants. The usual method for quantitation tests is by 'participant consensus'. It is obtained from the mean or median of the results from all participants or a proportion of the participants, e.g. the first 30 whose results are received, or from a selected group of participants who are designated as referees. For some tests, the target value is obtained by accurate measurement by expert reference laboratories using reference standards. In the IEQAS surveys the target values may be established from the UK NEQAS results when the same batch is used in both schemes.

Consensus Method: Mean or Median?

If the results from participants show a normal Gaussian distribution, calculate the mean and SD by the standard formula:

$$SD = \sqrt{\frac{(x - \bar{x})}{n-1}}$$

where

x = individual results;

$\bar{x}$ = mean of all results;

n = total number of results.

The data are then adjusted by deleting any which are 3 SD from the mean. The mean and SD (now termed 'weighted') are then recalculated.

If the results are spread out without an obvious Gaussian distribution, the median should be calculated instead of the mean and non-parametric statistical analysis should be used instead. An estimate of the measure of dispersion about the mean (i.e. SD) is obtained from the 50% spread of the distribution between 25% and 75%. SD is calculated as central 50% values (1.35). By this procedure, outlying results are automatically excluded in the calculation.

Analysis of Performance

Standard deviation limits

The SD is calculated by one of the methods described above. For this to be meaningful, there must be at least 15 participants in a set, and at least half the participants should have a sufficiently good performance in comparison with each other to avoid having a (weighted) SD which is too wide. The SD may be adjusted by one of the following methods:

Restrict analysis to a selected group of good performance

Use a predetermined constant CV from which the SD of the specific sample is calculated.

Use a predetermined SD based on clinical significance. Calculate a mean SD from pooled SDs for all samples in the previous six months, including the present survey.

A 'deviation index' (DI) or 'Z score' is then calculated for each participant. This indicates the difference between the individual laboratory results and the weighted mean of median and can be used to compare the performance of a laboratory with that of other laboratories as well as with its own performance in previous surveys.

The median should be used rather than the mean when there is an non-Gaussian distribution with a wide scatter of results. The deviation index score for any test may be interpreted as follows:

<1.0	satisfactory performance
1.0–2.0	still acceptable but borderline
2.0–3.0	requires review of techniques and check on calibration
>3.0	defect requiring urgent investigation

Other statistical methods

Methods based on CV and variance index are used extensively in clinical chemistry and can be applied to blood counts as well.

Assigned values and clinical significance

Results obtained from a group of expert laboratories may establish the correct values for various tests, especially if there are reference methods and standards available. The experts should establish their own interlaboratory precision, and thus determine the acceptable limits of SD.

Results from participants can then be judged by the extent of

Formula for calculating DI:

$$\frac{\textit{Actual result} - \textit{weighted mean or median}}{\textit{Adjusted SD}}$$

deviation from the assigned value, as follows:

<SD	excellent performance
1–2 SD	satisfactory
2–3 SD	need to check performance
>3 SD	serious problem requiring immediate attention

However, to base acceptable limits of performance on statistical analysis alone is unrealistic. It is also important to take account of the clinical significance of the test, and poor performance should be defined as a result which, if it had occurred with a patient's specimen, might have led to wrong clinical action to the detriment of the patient.

The acceptable limit of deviation from median or mean (whether established by experts or by consensus as described above) should also take account of unavoidable imprecision of the method used as well as normal diurnal variations.

Results within the following limits are usually considered satisfactory:

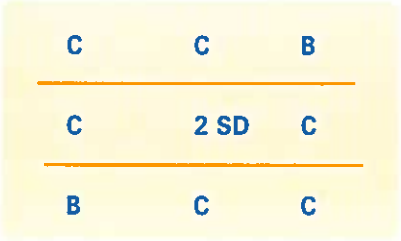
- Haemoglobin, RBC (by electronic counter) and PCV *4%–5%*
- MCV, MCH and MCHC *5%*
- WBC *10%*
- Platelet count *20%*
- Reticulocyte count *25%–30%* (by microscopy; low levels)

The advantage of this method is that it is valid even if there are few participants. The limitation is that there is no assurance that the assigned value is true and without bias. It is advisable for the centre to use several different techniques for each test, and preferably for the tests to be performed in three different laboratories which have been designated as reference centres because of their expertise.

Ratio analysis

Most surveys include two samples. The pairs may be random or selected to include two different levels of analyte concentration. The distribution of participants' results is plotted on the horizontal and vertical axes of a graph, from which it is easy to see whether errors are systematic or random.

If the test is satisfactory, results will lie in a central block comprising 2 SD for each set of measurements. Results in blocks B (figure below) indicate a consistent bias which may be incorrectly high or low. Results in blocks C indicate variable errors (inconsistency) in the measurements.



Quantitative and semiquantitative analysis

Qualitative tests will normally be recorded as Low/Normal/High, as +/++/+++, or simply as Positive/Negative. Numerical scores for each correct or incorrect observation should be weighted on the basis of their clinical and technical significance.

In morphology, results may be scored semiquantitatively by weighting the various features on the film, as agreed by referees, on the basis of their diagnostic significance or importance. Scoring may be positive, with correct observation being awarded a number of points and incorrect observations subtracted from the total, or negative, where good performance rates zero, and poor performance results in an increasingly high score.

Interpretation of results

In some tests, participants might be requested to indicate the significance of their results (e.g. 'normal', 'high', 'low'), taking account of information provided, such as age, sex and clinical state. Scoring should be similar to that for qualitative tests.

Non-participation

Failure to return results by the closing date in a survey should be regarded as 'unsatisfactory performance' or 'failed', and scored appropriately.

7. Protocol for Starting a National External Quality Assessment Scheme

Scheme in Haematology

Potential organizers of an EQAS should undertake a visit in order to understand the procedures and, at the same time, introduce the scheme to a small group of users who form the core of future participants. There should be at least 15 participants who should be conveniently situated to permit the delivery of samples without delay. Initially, only one analyte should be selected, possibly with two specimens at different concentrations.

For haematology, the obvious choice is haemoglobin in samples of whole blood.

From the experience of this trial it may be possible to increase the number of participating laboratories for a repeat trial. If necessary, increase the volume of blood collected. The volumes of ACD, antibiotics and ACD–saline solution added must then be increased proportionately.

Scheme in Haematology

1. Prepare 30 clean dry tubes with stoppers.
2. Collect 60 mL of normal blood into 9 mL of ACD. Add 105 units penicillin and 100 mg streptomycin. Mix well and then divide into two approximately equal portions and dispense into two conical flasks (labelled A and B).
3. Add 1.5 mL ACD to 10 mL saline (9 g/L NaCl) and add this solution to B.
4. Mix specimen A well, and with constant mixing pour approximately 2 mL volumes into 15 of the tubes. Stopper each tube tightly and label A.
5. Repeat fourth step with specimen B.
6. Prepare an instruction sheet and a report form. Enclose a copy of each with one sample of A and one of B in a package with suitable protective materials for postal transmission; send to each of 10–12 laboratories.
7. Keep one sample of A and of B for measurement of Hb in your own laboratory and send two samples of each to the WHO Collaborating Centre in London.
8. When results are returned calculate means and SDs. Also plot all results on a histogram. Show the reference values on the histogram as well.
9. Send a copy of the results to each participant and to the Collaborating Centre.

8 Preparation of Control Materials

Introduction

Methods for the preparation of control materials are discussed in this part. All laboratories should be able to make a lysate as well as preserve and stabilize blood specimens for IQC of blood counts.

The staff of a central laboratory should be able to make all the preparations for a NEQAS. In some situations, larger district general hospitals may be expected to provide EQA and IQC materials for smaller units, and also to be responsible for the control of haemoglobinometry in the health clinics of their area.

Accordingly, at least some laboratory workers will be expected to accept this responsibility and must thus be familiar with the preparation of QC materials. Organizers of training courses must be able to prepare small batches of the materials for use by the course participants.

General Notes

Human blood specimens used for calibration and control material for blood counts should be HBsAg and HIV and HCV antibody negative. Anticoagulated blood is usually available from blood transfusion services; the anticoagulant is either citrate phosphate dextrose (CPD) or

Preparation of Haemolysate

1. Centrifuge anticoagulated blood in bottles of appropriate size (e.g. 30 mL screw-cap glass containers). Remove the plasma and buffy coat aseptically.
2. To each red cell deposit add an excess of physiological saline (9 g/L NaCl), mix well, and recentrifuge. Discard the supernatant and any remaining buffy coat.
3. Repeat the saline wash twice to ensure complete removal of plasma, white cells and platelets, each time removing the top layer of packed red cells.
4. To the washed cells add half their volume of carbon tetrachloride, cap the containers and then shake vigorously on a mechanical shaker or vibrator for one hour. Take great care when using carbon tetrachloride as it is a hazardous chemical. Refrigerate overnight to allow the lipid/cell debris to form a semi-solid interface between carbon tetrachloride and lysate.
5. On the following day centrifuge at about 2500 *g* for 20 minutes. Remove the upper lysate layers and pool them in a clean bottle.
6. Using Whatman No. 1 filter paper in a Buchner funnel, filter the pooled lysate into a side arm flask connected to gentle water pump suction.
7. Repeat filtration using Whatman No. 42 filter paper. Change the paper if filtration slows down. It is important not to overload the funnel with the lysate.
8. To each 70 mL of lysate add 30 mL of glycerol. This stock material may be stored at 4 °C until required for dispensing.
9. If a lower concentration is required, add an appropriate volume of 30% (v/v) glycerol in physiological saline (9 g/L NaCl) to the concentrated lysate. Mix well.
10. Dispense aseptically into sterile containers with continuous mixing. Cap and seal.
11. To assign values for the Hb concentration, the ICSH reference method described in the WHO document[4] should be followed. The CV should be less than 2%. The product should maintain its assigned value for several months when stored at 4 °C.

acid citrate dextrose (ACD-NIH A). For lysates, blood in other anticoagulants (e.g. EDTA or heparin) can also be used. Care should be taken at all stages in the following procedures to avoid contamination. Where possible, sterile glassware and reagents should be used and aseptic handling procedures observed. Broad spectrum antibiotics may be added to aid sterility, e.g. 1 mega unit of penicillin and 1 g of streptomycin per 150 mL of blood has been found to be satisfactory.

Preparation of Preserved Blood

1. Human blood from donors of the same ABO blood group, negative for hepatitis B and C and HIV is collected in blood collection bags containing CPD or ACD. Equine blood may be collected in bags with a capacity of up to two litres.
2. Run the blood through blood administration sets directly into a round-bottom flask, mixing at the same time, and continue to mix for at least 20 minutes after the addition of the last unit of blood or other material.
3. Cell levels may be adjusted, as follows.
   - To increase the red cell count—sediment cells over exit vents of bag and run into the flask with a minimum of plasma.
   - To lower the red cell count—add a solution of anticoagulant in physiological saline (9 g/L NaCl); the anticoagulant–saline ratio must be the same as the usual anticoagulant–blood ratio.
   - To lower the white cell count—pass blood through a leucocyte filter.
   - To increase the white cell count—add fixed avian cells (see Preparation of 'pseudo-white' cells, page 187).
4. Add antibiotics (see paragraph on antibiotics in General Notes, above).
5. Dispense in sterile containers with continuous mixing; cap and seal. Refrigerate at 4 °C until needed.
6. For analysis, the sample should be gently mixed on a roller mixer or by hand before opening. Unopened vials of human blood keep in good condition for about three weeks at 4 °C, and those of equine blood are good for up to two months.
7. Methods for assigning values

   Packed cell volume by micro-haematocrit method on five samples from each of two vials from the batch in accordance with the procedure described in the WHO/CDC Anaemia manual[2]. The CV should be less than 2%.

   Red and white cells by electronic counting at least ten replicate counts on each of two vials from the batch by calibrated electronic counter; dilution by precalibrated pipettes. The CV should be less than 3%.

Preparation of Stabilized Whole Blood Control

Reagent

Formaldehyde (37%–40%)	6.75 mL
Glutaraldehyde 50%	0.75 mL
Trisodium citrate	26 g
Distilled water	100 mL

Method

1. Obtain preserved whole blood in CPD or ACD. This should be as fresh as possible and not more than 48 hours old. Filter through a 40 mm blood filter and measure the volume.
2. Add 1 volume of the reagent to 50 volumes of the blood.
3. Add antibiotics (see paragraph on antibiotics in General Notes)
4. Mix continuously on a magnetic stirrer for one hour at room temperature, then leave to stand overnight at 4 °C.
5. To obtain different values, remove 50 mL of supernatant plasma and keep in reserve.
6. Remix the remainder. Take a part of the stock and dispense 2 mL aliquots with continuous mixing into sterile containers.
7. Return the plasma (see No. 5) to the remaining stock, remix and dispense the rest of the stock in the same manner (see No. 6).
8. Cap and seal; refrigerate at 4 °C until needed.
9. For analysis, the sample should be gently mixed on a roller mixer or by hand before opening. Keep unopened vials in good condition for several months for all blood count parameters.
10. To assign values:

    Hb (see No.11 on page 184: Preparation of haemolysate)

    RBC (see No.7 on page 185: Preparation of preserved blood)

    WBC (see No.7 on page 185: Preparation of preserved blood)

    Platelets

    Haemocytometry (with phase contrast microscopy, if available) five replicate counts on two vials from a batch.

    PCV obtained by centrifugation (see No. 7 on page 185: Preparation of preserved blood) is likely to differ from the measurement of the same sample by a blood cell counter.

Preparation of Fixed Cells ('Pseudo-White' Cells)

Chicken and turkey red blood cells are nucleated and when fixed, their size, as recognized by electronic cell counters, is within the leucocyte size range. Thus, they are suitable to act as 'pseudo-white' cells in preserved whole blood.

Similarly, human and animal blood can provide cells of various sizes. For use as a WBC control, it is sufficient to collect 25 mL of blood into any anticoagulant and process as follows before adding to a bulk of preserved blood.

Reagents

I. ***0.15 M iso-osmotic phosphate buffer (pH 7.4)***

A. *23.4 g/L sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$)*

B. *21.3 g/L anhydrous disodium hydrogen phosphate (Na_2HPO_4) or 53.7 g/L $Na_2HPO_4.12H_2O$*

Both stock solutions keep well when refrigerated.

For use, mix 18 mL A with 82 mL B. The pH should be adjusted to 7.4.

II. 0.25% glutaraldehyde fixative

To 1 L of phosphate buffer, add 5 mL 50% glutaraldehyde solution (commercially available). Mix and use at once.

Method

1. Centrifuge the blood and remove plasma.
2. Add an excess of phosphate buffer to the red cells, mix and transfer to a centrifuge bottle. Recentrifuge and discard the supernatant and buffy coat.
3. Repeat the washing and centrifugation steps twice.
4. To the washed cells add ten times their volume of glutaraldehyde fixative, mix by vigorous shaking to ensure complete resuspension and rotate slowly on a mechanical mixer for one hour. To test for complete fixation, centrifuge 2–3 mL of the suspension, discard the supernatant. Add 2–3 mL water to the deposit, mix and centrifuge. If haemolysis occurs, fixation is incomplete, and either more time is needed or the stock glutaraldehyde should be replaced.
5. When fixation is complete, centrifuge the suspension and discard the supernatant.
6. Add an excess of distilled water to the fixed cell deposit, resuspend and mix by stirring and shaking. Again centrifuge and discard the supernatant. Repeat twice.
7. Resuspend the washed fixed cells to approximately 30% concentration in physiological saline (9 g/L NaCl) with 0.1% sodium azide. Mix well by vigorous shaking.

Preparation of Fixed Cells/'Pseudo-White' Cells (contd.)

8. Carry out a rough count by a routine method to determine the approximate concentration.
9. If it is intended to be used as a stock, autoclave at 15 psi (121 °C) for 15 minutes and then store at 4 °C. If aliquots are to be removed subsequently by opening the capped container, it is advisable to add antibiotic (see General Notes on page 184) and then store at 4 °C.
10. If it is to be used as a WBC surrogate, mix the stock as described above and then transfer an appropriate amount to a volume of preserved blood (see section on Preparation of preserved blood, page 185) from which the leucocytes have been filtered.
11. Mix well for 20 minutes and dispense into sterile containers with continuous mixing. Cap and seal.
12. Before use, resuspend by vigorous hand shaking (or by a vortex mixer) until no clumps remain at the base of the container, then roller mix for at least 20 minutes. If available, sonication for 1–2 minutes is also helpful.
13. To assign WBC values, at least ten replicate counts from each of two vials from the batch should be obtained using a haemocytometer, diluting by precalibrated pipette. The CV should be less than 5%. At 4 °C, the shelf-life will be several months.

Preparation of Fresh Platelets

1. Obtain 50 mL of fresh platelet concentrate in a blood donor pack.
2. Centrifuge at 1000 *g* for 10 minutes and discard the supernatant.
3. Wash the deposit in phosphate buffer (see section on Preparation of fixed cells, page 187) and suspend in 2 mL of the buffer.
4. Add 10 mL of glutaraldehyde fixation (see section on Preparation of fixed cells, page 187), shake vigorously to ensure complete resuspension and then mix in a mechanical mixer for one hour.
5. Centrifuge, discard supernatant and wash deposit twice in the buffer; resuspend in 10 mL of CPD (see section on Preparation of preserved blood, page 185)
6. Carry out a rough platelet count to determine the approximate concentration. Add the appropriate amount to the preserved blood (see section on Preparation of preserved blood, page 185) and repeat steps 5 and 6 in section on Preparation of preserved blood, page 185.
7. Method for assigning value: haemocytometry (with phase contrast microscopy, if available) on 5 replicate counts on two vials from the batch; dilution by precalibrated pipettes. The CV should be less than 10%.

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Chapter 5

Quality Assurance in Blood Banks

1. Introduction

THE PRACTICE OF quality assurance (QA) in blood bank technology has become very important with the advent of AIDS. This is even more important in countries of the Region where the number of blood banks has increased phenomenally in the past few years.

Quality assurance in blood banks is in two main areas—blood group serology and testing for bloodborne infections. With the explosion of AIDS in countries of the Region, the QA of testing methodology of bloodborne infections, especially for HIV antibody, HBsAg, HBV antibodies and HCV antibodies has become absolutely essential.

Blood group serology and testing for bloodborne infections are the two main components of quality assurance in blood banks.

As the External Quality Assessment Scheme (EQAS) for microbiology will cover the QA of bloodborne infections, it is suggested that the same organizer provides a scheme for blood banks. Details of such EQA are provided in Chapter 3, 'Quality Assurance in Microbiology'.

2. Quality Assurance Programme in Blood Banks

THE INTRODUCTION OF a quality assurance programme (QAP) in a blood transfusion centre requires:

- In-depth vision and knowledge of all the aspects of blood transfusion,
- Planning for effective donor selection, donor screening, laboratory work, issue of safe blood and transfusion of blood to the recipient, and
- Formulating the mechanism for monitoring to ensure that quality is maintained.

Total QA includes:

- Quality in procurement (donor, material, reagent).
- Quality in preparation (efficient and effective blood component preparation).
- Quality in design and development (improved techniques and procedures).
- Quality in supply (transportation and service).
- Standardized methodology for screening of blood.
- Participation in EQA of blood group serology and serology of bloodborne infectious agents.

3. Internal Quality Control (IQC)

AN INTERNAL QUALITY control programme includes:

- Proper identification of all samples. This is of extreme importance as the majority of ABO incompatible transfusions are due to clerical/documentation/identification errors.
- Validation of the sensitivity, specificity and reproducibility of new batches of test kits and reagents.
- Strict adherence to the recommended procedure.
- Use of the appropriate test and internal controls.
- Regular proficiency testing exercise.
- Adherence to safety guidelines and safe disposal.

Standard Operating Procedures (SOPs)

An SOP is in the form of a booklet providing a complete set of instructions to perform a certain task and contains an accurate written description of a working procedure.

Quality Control of Equipment

Besides the assessment of functions immediately after installation and repair of equipment, day-to-day checks in the routine use of equipment are necessary. For effective QC of equipment, the following principles must be strictly adhered to:

- All the equipment in the blood transfusion laboratory should meet mandatory specifications.
- A written record of periodic function checks and maintenance on each piece of equipment should be mandatory.
- A preventive maintenance should be planned for trouble-free operation.
- Uninterrupted power supply should be maintained for all the equipment with efficient back-up system.
- Annual maintenance contract with manufacturers and suppliers should be obtained.

SOPs to be prepared and used in blood transfusion centres

- Donor selection and blood collection.
- Red cell serology including techniques such as ABO and Rh grouping, compatibility testing, antiglobulin test, etc.
- Screening of donor units for syphilis, HIV, HBsAg, HCV, VDRL, etc.
- Reagent preparation.
- Pretransfusion testing.
- Preparation of blood components.
- Issue of blood.
- Transportation of blood.
- Transfusion of blood to a patient (for use in OT/Ward).

Quality Control of Reagents

The primary objective of quality control of a reagent is to ensure that the reagent is functioning as expected. The following principles apply to the storage of reagents:

- Out-of-date reagents should never be used.
- The oldest reagents should be used first (first-in-first-out).
- There should be a system for indicating when supplies run low.
- Every new lot of reagents should be evaluated for potency and specificity. The results should be recorded.
- A low potency, contaminated or defective reagent should never be used.

Quality Control of Techniques

Quality control of techniques is important to ensure a consistently high level of reproducibility and performance of most commonly used techniques, such as ABO and Rh(D) grouping, antihuman globulin test, compatibility testing and antibody screening. It is very important to use negative, positive and autocontrols to assess the validity for accuracy and sensitivity of the test.

4. External Quality Assessment Programme in Blood Group Serology

Scope

IDEALLY, AN EQA programme should be set up for all areas of blood group serology involved in pre-transfusion and antenatal serology. However, it may be preferable to start with the most important tests and gradually include further tests as the scheme is established and if the number of potential participants warrants it.

ABO grouping, Rh(D) grouping and cross-matching are the most important and should be established first. Screening for, and identification of, irregular antibodies should be included at a later date for those participants who routinely perform these tests.

Analytes

Analytes include:

- ABO and Rh(D) grouping
- Antibody detection
- Cross-matching
- Antibody identification (for suitably registered participants).

Aspects of Performance Assessment

Participants are required to interpret their results for:

1. ABO and Rh(D) grouping
2. Antibody detection
3. Cross-matching
4. Suitably registered participants are required to identify the antibody specifications present in samples with a positive antibody detection screen.

Minor exercises are confined to antibody detection, together with antibody identification.

Materials Distributed

The materials distributed in an EQA programme in blood group serology include human red cells and defibrinated plasma.

EQAS should be set up in blood group serology in pre-transfusion and antenatal serology.

Number of Exercises Within an Year

There should be four major exercises per year at three-monthly intervals, covering all routine aspects of blood group serology.

The dates of dispatch of the exercise should be notified in advance to participants.

Registration for the Various Sections of the Scheme

Participants should register for the sections which cover the work routinely undertaken in their laboratory.

Dispatch of Exercise Material

Exercise materials should be dispatched by first class mail. Exercises are sent by air mail to distant participants, unless other means of delivery are specifically requested or are essential to ensure the viability of the test material.

The nature of the contents of the package, the storage temperature on receipt and the address of the sender are indicated on the package as well as the 'Non-Infectious Perishable Biological Substance' sticker.

The dates of dispatch are notified in advance to participants. Participants who fail to receive an exercise within two weeks of the dispatch date should notify the organizing laboratory.

All exercise material is addressed to the technologist in the transfusion/ haematology department.

Preparation and Pre-acceptance Testing of Material

General

1. Cells and sera used for EQA material are best prepared from normal blood donations which have been tested and found negative for infectious diseases, in line with the country's policies.
2. Sera and red cell pools (see section on Red cells—source, adjacent column; and see section on Serum—source) should preferably be dispensed under aseptic conditions. Samples of each pool need to be checked for bacterial contamination prior to and after bottling.
3. ABO-matched pairs of sera and cell pools should be used.
4. Pre-bottling serology should be performed to ensure that the processed material is giving the expected reactions (see section on Serology).
5. Each pool should be dispensed into bottles, and capped and labelled discretely to prevent any mix-up between pools. Each sample should be labelled with a unique exercise identifier and a pool identifier, e.g. 98/01 patient A. Tests should be performed post bottling to ensure that each pool has been labelled correctly.

Red Cells

Source

Red cells used to represent a single patient or donor should ideally be taken from a single donation and diluted to provide sufficient material for all participants. Otherwise, red cells of identical ABO, Rh(D) and any other antigens relevant to the exercise should be pooled, thoroughly mixed and diluted to provide a homogeneous sample for all participants. The final dilution, whether derived from a single or pooled source, is referred to subsequently as the 'red cell pool'.

Viability

The red cells must survive the postal service and storage throughout the duration of the exercise. A diluent such as modified Alsevers including antibiotics helps to maintain the viability of cells and prevents bacterial contamination for up to 40 days at 4 °C.

Postage time and the closing dates should be kept to a minimum. Where possible, red cells should arrive within a maximum of seven days after posting and a further week could be allowed for the closing date.

Serum

Source

Serum or defibrinated plasma can be used. Ideally, single donations, or pooled donations of identical ABO group, containing weak antibody should be used to best represent patient samples.

If insufficient material is available, stronger antibodies may be diluted in ABO identical serum or defibrinated inert plasma. The final dilution, whether derived from a single or pooled source, is referred to subsequently as the 'serum pool'.

Viability

The final serum pool should be sterile, filtered and sodium azide may be added to aid sterility. Residual fibrin may cause problems during testing. Therefore, fibrinogen measurements should be performed on the final pool prior to bottling.

Antibodies must not be dependent on the presence of complement for their activity.

Serology

The matched pairs of sera and cells should be grouped for ABO and the cells for Rh(D). It is also desirable to include an auto by both direct agglutination at room temperature and by Indirect Antiglobulin Test (IAT). The 'donor' red cell pools need testing for ABO, Rh(D) and any other antigens relevant to the exercise. A direct antiglobulin test which should be negative on the 'donor' red cell pools should also be performed.

If the serum is only used for reverse grouping and cross-matching, only these procedures should be performed prior to bottling and dispatch. The crossmatch should be performed by IAT using a satndard tube technique. Direct agglutination tests should also be included at 4 °C to exclude the presence of unwanted cold IgM antibodies.

Where participating laboratories commonly use further techniques or technologies, e.g. enzymes, albumin, column agglutination technology, these should be included in the repertoire of testing performed prior to dispatch.

If antibody screening and identification are included in the scheme, more extensive testing is required.

Diluent serum/plasma and inert pools must be tested against appropriate cells to ensure that they are free from irregular antibodies.

Depending on availability of red cells, it may be desirable to include some low frequency antigens. Potency tests (i.e. titres) should be performed throughout the exercise to ensure the stability of the material.

The final serum pool should be sterile, filtered and stored with sodium azide to keep it sterile.

Documentation

Details and results of all source material, processing and testing should be documented.

Post-distribution Testing

The organizing laboratory must ensure that the material is viable throughout the timescale of the exercise. To achieve this, a set of samples, identical to those dispatched to participating laboratories should be posted to the organizing laboratory. Tests should be performed on receipt of the samples and on the last working day prior to the closing date. It may also be useful to test samples for the maximum length of time that the samples would be in the post to the furthest participant.

Exercise Design

General

The exercises are best designed to give the maximum number of tests using the minimum number of samples. The best way to achieve this will depend on the routine practices of the participating laboratories and on the availability of material.

The number of 'patient' and 'donor' samples distributed within any exercise can be flexible, but at least two of each should be distributed to enable the assessment of sample transposition and transcription errors. The following examples illustrate this.

Example 1

Two 'donor' red cell samples for ABO and Rh(D) grouping and cross-matching against two 'patient' serum samples. Two additional 'matched' donor serum samples would be required for reverse grouping. Overall sample requirements are two red cells and four sera. The advantage of this is that ABO incompatibilities can easily be arranged.

Example 2

Two matched pairs of 'patient' red cells and sera for ABO and Rh(D) grouping and crossmatching against two 'donor red cell samples'. Overall

sample requirements are four red cells and two sera. This would probably be more representative of the clinical situation in laboratories where donor units are brought in from elsewhere and not routinely grouped in the laboratory prior to transfusion.

If ABO incompatibilities are to be assessed, the participating laboratories would have to assume that all 'donors' are nominally ABO compatible with all 'patients' and not regroup them.

ABO and Rh(D) grouping

It is desirable to include samples of different blood groups within one exercise so that sample transposition errors can be detected.

Cross-matching

Each exercise should contain a mixture of compatible and incompatible crossmatches.

Antibody screening and identification

Samples for antibody screening (the same as those distributed for crossmatching) should be a mixture of those containing weak irregular antibodies of potential clinical significance and inert samples. If antibody identification is also included, single and dual specificities should be included, depending on the reagent red cells available to the participating laboratories.

Correct Results/Target Values

Unlike quantitative schemes, where a consensus value may be used, the results for blood group serology are either right or wrong (positive or negative). The 'true' or correct result may be that recorded by the organizing laboratory in conjuction with one other 'reference' laboratory. This 'reference' laboratory may be the supplier of the material, in cases where the supplier is different from the organizing laboratory.

All serology must be performed by the organizing laboratory prior to the dispatch of samples and on the last working day prior to the closing date.

Apparent false-positive results submitted by participating laboratories for antibody screening will need further investigation to exclude the presence of antibodies to low frequency antigens.

Performance Monitoring

Each 'analyte' should be assessed separately, including non-return of results (or late results received after the closing date, giving due consideration to distant laboratories). Scoring can be positive or negative, with points awarded for the correct results or penalty points for errors.

There should be a definition of unsatisfactory performance, based on the number of points accumulated over a set number of exercises. The scores should reflect clinical significance of the error, e.g. a major ABO grouping error should be more heavily penalized than a false-positive cross-match.

Wherever possible, repeat samples should be available to those laboratories which have made errors. Unsatisfactory performers should be contacted and an attempt made to elucidate the source of the error.

Appropriate advice regarding techniques and procedures should be given where possible. Training may be necessary either at the participant's site or at the organizing centre.

Trends in performance relating to the use of particular techniques or reagents should also be monitored. This will require the regular distribution of questionnaires or the supply of standard information at the time of registration or with each exercise.

Reports

A report should be distributed to each participating laboratory as soon as possible following the closing date, indicating the participant's results and scores and also the correct results. If possible, the overall results for the country should be included,

otherwise these and any trends in performance can be distributed later.

Laboratory Performance

The participating laboratory should handle samples as closely as possible to clinical samples. They should be subject to the routine procedures and performed by a member of staff who performs the work routinely. The results can of course only give a snapshot of events at the time and may reflect the performance of the hardware or reagents used, or the technical skill of the individual performing the tests. It may be useful for any excess sample to be used for internal QC, involving additional staff, after the results have been returned to the organizing centre.

Training of poor performers must form an integral part of EQAS.

As in all the EQASs the training of poor performers must form an integral part of the NEQAS. The training programme should be prepared by the Advisory Committee of which the organizer of the scheme is also a member. Training may be carried out regularly. Training may also be imparted at site. The Advisory Committee should meet at least once an year to evaluate the scheme and future programmes, and also liaise with accreditation authorities.

Part IV

Biosafety in Laboratories

1. Introduction

EACH LABORATORY should consider the prevailing circumstances and work out its own strategy to prevent accidents while handling infectious materials.

Irrespective of the level of the laboratory, certain precautions must be taken everywhere while handling infectious material. Even if the laboratory is not responsible for processing a specimen and is only involved in its transport, it is desirable to avoid all mishaps with potentially dangerous pathogens.

Each laboratory worker must be familiar with the risks involved and with the mandatory steps that must be taken to avoid accidents. The rapid spread of HIV has challenged laboratory workers to exercise caution while conducting different laboratory tests and handling potentially infectious material.

Each laboratory has to have its own strategies to prevent accidents while handling infectious materials.

Laboratory personnel should be familiar with terms in frequent use as well as the risk grades of different microbes, the four biosafety levels of laboratory and biological safety cabinets.

The individual worker can prevent accidents by following safety rules while working in a laboratory, handling infected materials in the field or at the bedside of the patient.

Complications can be reduced through a systematic approach to each event.

The disposal of infected material depends upon the disposability or otherwise of the material and the nature of pathogen.

The shipment of specimens containing potentially infective microorganisms can be challenging where facilities for packing and transportation are poor.

The rapid spread of HIV has challenged laboratory workers to exercise caution while conducting laboratory tests and handling potentially infectious material.

Definitions

The following are some commonly used terms in biosafety.

Biosafety

Biosafety includes every activity related to safeguarding a population from the biologically untoward effects of infectious agents. In the context of health laboratories, biosafety aims to protect all those who are exposed, directly or indirectly, to infectious agents while handling laboratory specimens.

Biosafety level of risks with organisms

Microorganisms are classified into hazard groups at different levels on the basis of risks to laboratory staff, spread in the community, pathogenicity and availability of effective prophylaxis and treatment.

Risk Group 1

Organisms are harmless or pose a minimal hazard to laboratory staff and the community.

Risk Group 2

Organisms are a moderate potential hazard for laboratory staff but only a limited risk for the community. Effective preventive measures and treatment are available.

Risk Group 3

Organisms cause serious human disease and pose serious hazards to laboratory staff. They do not ordinarily spread from one infective individual to another and are, therefore, a low risk for the community. Effective prophylaxis and treatment are normally available. The organisms are transmitted through aerosol, autoinoculation or ingestion.

Risk Group 4

Organisms cause severe human disease and are a high risk for laboratory personnel. They readily spread from one infected individual to another in the community. There is no effective treatment or prophylaxis and they require maximum containment facilities during handling.

Biosafety Levels

Microbiological laboratories are divided into four levels. Level 1 is intended for work with organisms of lowest risk and level 4 designated for work with organisms of highest risk.

Biosafety Level 1

Work at this level involves microorganisms which are not known to cause disease in healthy adult humans. Facilities should be adequate for teaching and microbiological techniques should be good. Work is conducted on open benches with no special containment equipment.

Biosafety Level 2

This class of laboratory is suitable for work involving agents of moderate potential hazard to staff and the environment. Access to the laboratory is limited. While at work, staff take universal precautions and follow good microbiological techniques. Procedures which create infectious aerosols are performed in biological safety cabinets or within some other physical containment facilities.

Biosafety Level 3

This level of laboratory includes clinical, diagnostic, teaching and research facilities and works with agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation. All procedures are conducted within biological safety cabinets or other physical containment devices by personnel wearing appropriate protective clothing or devices.

Biosafety Level 4

This laboratory is needed for organisms that cause life-threatening diseases. Apart from the facilities available at Level 3, an airlock entry, a shower exit, and special waste disposal facilities should be provided at Level 4. Class III biological safety cabinets, positive pressure suits, double-ended autoclaves and filtered air are essential safety requirements.

The double-ended autoclave would usually open from a class III safety cabinet line into a lobby outside the room. Liquid wastes from the laboratory are taken to holding tanks for heat sterilization or chemical disinfection before discharge into the sewers.

Biohazardous Waste

Waste products, including body fluids and tissues, that may carry dangerous human pathogens constitute biohazardous wastes. They originate from health care facilities and/or research laboratories. A relatively small or confined group of people are at risk of acquiring infection during the time necessary for the infective agent to dissociate or otherwise become inactive. Biohazardous wastes are recognized by an internationally recognized symbol.

Containment

The confining or prevention of dissemination of a potentially hazardous agent constitutes containment. Containment is of two types.

Primary containment involves the protection of personnel and the immediate environment from hazardous agents by the use of safety equipment, biological agents and vaccines. *Secondary containment* involves protection of the environment external to a 'contained' area which is provided by a combination of facility design and operational practice.

2 Biological Safety Cabinets

BIOLOGICAL SAFETY cabinets are used to protect laboratory workers from aerosols generated during microbiological manipulations. Dangerous aerosols in the air are removed by high-efficiency particulate air (HEPA) filters.

Classes of Biological Safety Cabinets

There are three classes of biological safety cabinets.

Class I Cabinets

These cabinets protect the worker by the flow of air which moves inwards from the room and over the working surface.

The direction of airflow ensures that dangerous agents stay within the cabinet. The air exits through a HEPA filter to prevent the discharge of microorganisms into the environment. Such cabinets protect the operator but do not protect the material within the cabinet from contamination. They may be used with organisms belonging to Risk Groups 2 and 3.

Class II Cabinets

These cabinets protect both the laboratory worker and the material within the cabinet. Room air is drawn in at the front by a fan unit under the working surface, which passes the air between two HEPA filters so that clean air is directed on to the working space. Thus, a clean environment is provided. Pathogens are prevented from escaping into the room by the inward flow of air, the two HEPA filters and the exhaust system.

Class III Cabinets

These cabinets are totally sealed and ventilated, and arm-length, impermeable gloves are employed for manipulations. The airtight cabinet is maintained at negative air pressure. The supply air is HEPA-filtered and the exhaust air is passed through two HEPA filters in series. Thus, a barrier between the operator and the material is provided and the cabinets are used for Risk Group 4 organisms.

Material is taken out through a double-door autoclave, and through a second air lock where decontamination procedures may be carried out.

Class I cabinets protect only the operator. Class II cabinets protect both the operator and the material. Class III cabinets protect the operator, material and environment from getting contaminated.

Regular Testing and Monitoring of Safety Cabinets

The safety cabinets must be regularly tested for airflows, filter integrity, containment (operator protection), external contamination, cross contamination and leakages.

Gas should be avoided in safety cabinets as heat distorts airflows. Gas and electricity cut-off points should be near but outside the safety cabinets.

Decontamination of Safety Cabinets

Aerosols during work contaminate the inside surfaces and filters. The working surface and walls should be swabbed after work preferably with glutaraldehyde, as phenolics may leave sticky residues and hypochlorites are corrosive.

For thorough decontamination (after spillage, before maintenance, filter changing and testing) fumigation is recommended. Fumigation can be done with formalin either by

boiling formalin or heating para-formaldehyde which is a solid polymer.

The prefilter should be removed from inside the cabinet and placed on a working surface. The front closure and all service holes of the cabinet must be sealed with masking tape. The quantity of formalin can be calculated by multiplying 60 mL with the area of the cabinet in cm^3. This quantity is placed on the heater in the cabinet. The heater is switched on and formalin boiled. When about half of the formalin has boiled away, the fan is run for about 15 seconds to bring the formaldehyde into the filters. After switching off the heater the cabinet is left closed overnight.

Fumigation is the recommended procedure for decontamination of biosafety cabinets.

Next morning the cabinet fan is switched on and then the front closure is opened very slightly to allow air to pass in and purge the formaldehyde from the cabinet. After several minutes the front closure is removed and the cabinet fan allowed to run for about 30 minutes. Any obvious moisture remaining on the cabinet walls and floor may then be wiped off.

3. The AIDS Pandemic and Safety of Laboratory Workers

THE AIDS PANDEMIC is a challenge for laboratories at all levels since they are involved in screening prospective blood donors and patients with AIDS.

The collection of blood samples and their transfer within the laboratory could be risky for the patient, health worker, transport worker and laboratory personnel if universal precautions are not strictly followed. Not only the technical laboratory staff, but the support staff, including transport and postal staff, could be at risk.

Acquired infection occurs primarily from contamination of hands and mucous membrane of the eyes, nose and mouth by infectious blood and other body fluids.

Collection of blood samples and their transfer within the laboratory is risky for patients, laboratory staff, support staff, the health worker and transport staff if universal precautions are not strictly followed.

Table 1 lists the procedure, persons at risk and mode of transmission of HIV, HBV and other bloodborne agents. Current studies indicate that the HIV infection rate for laboratory workers is low.

The risk of HIV infection following percutaneous needle-stick exposure to HIV-contaminated blood is estimated to be 0.4%.

In contrast, the risk of HBV infection following similar exposure is 100 times greater.

The AIDS pandemic has been acompanied by the re-emergence of tuberculosis and special precautions must be taken to prevent the risk of infection besides observing safe work practices.

Table 1

Procedure, persons at risk and mode of transmission of HIV, HBV and other blood-borne agents

Procedure	Persons at Risk	Mode of Transmission
Collection of blood sample	Patient	Contaminated needles Contaminated hands or gloves of health worker
	Health workers	Skin puncture by needle or broken specimen container Contamination of hands by blood
Transfer of specimens (within laboratory)	Transport workers Laboratory personnel	Contaminated exterior of specimen container Broken container Spill or splash of specimen
HIV serology and virology		Skin puncture or contamination of skin or mucous membrane Contaminated exterior of specimen container Contaminated work surface Broken specimen container Perforated gloves
Cleaning and maintenance	Laboratory personnel	Skin puncture or skin contamination
	Support staff	Spills or splashes Contaminated work surface
Waste disposal	Laboratory personnel	Contact with contaminated waste
	Support staff	Puncture wounds and cuts
	Transport workers	
	The public	
Shipment of specimens (to other centres)	Transport workers Postal workers The public	Broken or leaking specimen containers

4. Dos and Don'ts

ALL LABORATORY personnel, including ancillary staff, should avoid the risk of exposure to hazardous organisms by observing the basic principles of personal hygiene while working in laboratories. Table 2 gives some dos and don'ts which laboratory personnel must observe during field visits to collect specimens or carry out simple laboratory test procedures. Nursing staff in wards must also be familiar with these instructions.

Table 2

Dos	Don'ts
Do keep your hair tied.	Do not eat or smoke or apply cosmetics in the laboratory.
Do wash your hands after completing your work.	Do not lick labels, but instead use gum or adhesive.
Do wear protective clothing, including gloves.	Do not touch your eyes, nose or other exposed parts while working with infected material.
Do disinfect the workplace after completion of every activity as well as at the end of every working day.	Do not pipette by mouth.
Do keep your nails trimmed all the time.	Do not wear loose clothes while in the laboratory or while engaged in handling infectious material anywhere.

5. Universal Precautions

UNIVERSAL PRECAUTIONS should always be taken when it is likely that the skin or mucosa (eyes, nose, mouth, respiratory tract) may come in contact with blood or body fluids. These precautions are based on the assumption that all blood and body fluids are potentially infectious and may contain HIV, HBV and other bloodborne microorganisms. Similarly, all equipment, surfaces and materials contaminated with blood or body fluids should be treated as potentially infectious.

The components of universal precautions are:

- Use of protective barriers (gloves, gowns/aprons, face-mask, goggles).
- Prevention of accidents, particularly injuries by sharps.
- Proper use of disinfection and sterilization techniques to render contaminated material, instruments and surfaces safe.
- Safe discard and disposal of contaminated waste.

Mandatory Day-To-Day Measures

Biosafety measures are meant to protect laboratory workers in diagnostic and research laboratories while handling material containing HIV as well as other infective agents.

- The international biohazard sign must be displayed on doors of laboratories where Risk Group 2 and higher-group microorganisms (e.g. HIV) are handled and access to the laboratory restricted. The symbol is red, orange, or black on a yellow background and reads 'Biohazard. No Admission'.
- Blood, body fluids and tissue samples should be collected and transported in sturdy containers with securely fitting screw-caps to prevent leakage. Care should be taken not to contaminate the outside of the container and laboratory form.
- All persons handling and processing blood, body fluids, infectious materials and infected animals must wear gloves. After use, gloves must be washed while on the hands and after removal. Gloves are then disinfected or autoclaved for disposal. All laboratories that work with material that is potentially infected with HIV should be provided with a generous supply of good-quality gloves.
- Gloves should be discarded whenever they are thought to have become contaminated, and hands washed before putting on new gloves.
- A laboratory gown or other protective clothing should be worn while working in the laboratory and removed before leaving the laboratory.
- The workplace should not be left, even to walk around the laboratory, while wearing gloves.
- Hands should be washed with soap and water immediately after any contamination is encountered and after work is completed.
- Perform all technical procedures in a way that minimizes the risk of generating aerosols, droplets, splashes, or spills.
- For routine histological, haematological, serological and microbiological work, a microbiological safety cabinet is not necessary. But procedures such as blending, sonicating and vigorous mixing may generate droplets and should be carried out in safety cabinets (Class I or II).
- Special precautions for tuberculosis—all sputum samples should be processed in containment (biosafety Level 3). Extrapulmonary samples for tuberculosis may be processed in a biosafety Level 2 laboratory and positive cultures can be dealt with in containment (biosafety Level 3).
- The laboratory should be kept clean, neat and free from unnecessary materials and equipment.
- Take precautions to prevent puncture wounds, cuts, and abrasions and protect existing wounds, skin lesions, conjunctiva and mucosal surfaces. Whenever possible, the use of needles, and other sharp

instruments should be avoided; used needles, syringes and sharps should be placed in a puncture-resistant container; and used needles should not be recapped or removed from syringes.

- Do not touch the eyes, nose, mouth and other exposed parts of the body with gloved or ungloved hands.
- Never pipette out by mouth.
- Work surfaces should be disinfected when work is completed and at the end of each working day. Hypochlorite solution with a minimum concentration of 0.1% available chlorine (1g/L, 1000 ppm) serves as an effective all-purpose disinfectant.
- Safely discard and dispose of contaminated waste.
- An effective insect and rodent control programme should be ensured.
- Needle-stick or other puncture wounds, cuts, and skin contaminated by spills or splashes of specimen material should be thoroughly washed with soap and water; bleeding from any puncture wound should be encouraged and covered with a waterproof dressing.
- If blood/body fluid splashes into the mouth, eyes or nose, wash thoroughly with plenty of running water by rinsing, and splashing.
- All accidents and exposures to infectious material should be reported immediately to the laboratory supervisor, and appropriate medical evaluation, treatment and counselling provided.

Decontamination of Spills

Decontamination of small spills with low-to-moderate risk microorganisms is carried out as follows:

- The affected area is flooded with an appropriate disinfectant (sodium hypochlorite 5 g/L, 5000 ppm available chlorine) and covered with paper towels. Leave it for 30 minutes.
- Other workers are warned to avoid this area.
- An autoclavable dustpan and forceps are used to pick up paper towels and any solid materials with gloved hands and put in a bag. The area is swabbed with fresh disinfectant.
- Paper towels, dustpan and forceps are decontaminated by autoclaving.

Large volumes of spills (e.g. when culture tubes/petri dishes are dropped on the floor and break) or highly pathogenic agents require special measures. These should be immediately reported to the laboratory supervisor so that appropriate action is taken.

Guidelines for Collection of Blood Samples

The major hazards to people taking blood specimens are contamination of the hands while drawing blood, and penetrating injuries caused by needles. To minimize such accidents:

- Gloves should always be worn while taking blood.
- If blood spills on the gloves, they should be discarded.
- Care should be taken to avoid contamination of the hands while taking blood.
- Hands should be washed with soap and water immediately after any contamination with blood and after work is completed.
- Laboratory gowns should be worn.
- Used needles and syringes should be placed in a puncture-resistant container; used needles should not be recapped, nor should they be removed from syringes.
- Specimen containers should be sealed securely, and the outside of the container should be wiped clean of blood contamination with disinfectant.
- In the event of needle-stick or other skin injuries, the wound should be washed thoroughly with soap and water and bleeding should be encouraged.

6. Supplementary Guidelines for Serological Laboratories

HIV-CONTAMINATED MATERIAL should be handled in a separate laboratory, developed exclusively for such work. If this is not possible, a secluded and clearly identified working area should be provided within the laboratory.

HIV-contaminated material should always be handled in a laboratory or a clearly defined work area developed specially for such material.

Biological safety cabinets are not required for serological testing of potentially HIV-contaminated material, but the walls, ceilings and floor of the laboratory should be smooth, easy to clean, impermeable and resistant to the chemicals and disinfectants normally used in the laboratory. The floors should be non-slippery. The bench tops should be impervious and resistant to disinfectants, acids, alkalis, organic solvents, and moderate heat, and the laboratory furniture should be sturdy and easy to clean.

Wash-basins should be provided in each laboratory, preferably near the exit. Doors to the laboratory should be self-closing and have vision panels, and windows should be fitted with fly screens.

An autoclave for decontamination of infectious laboratory material and waste should be available in the same building as the HIV laboratory. Facilities for storing outer clothing and personal items and space for eating, drinking and smoking should be provided outside the workroom.

7. Decontamination

WHEN AN AUTOCLAVE is not available for decontaminating equipment, etc. a pressure cooker should be used at the highest possible pressure. The use of boiling water for 30 minutes is also effective for decontamination.

Appropriate concentrations of sodium hypochlorite, formaldehyde or glutaraldehye are desirable for disinfection. Sodium hypochlorite is used universally as a disinfectant. Stock solution is diluted to 1 g/L (1000 ppm), or to 5 g/L (5000 ppm), for disinfection of blood spillages and organic matter.

Formaldehyde is useful for disinfection of rooms and equipment at temperatures above 20 °C and 70% relative humidity. Formaldehyde is generated by heating paraformaldehyde (10.8 g/m^3) or by boiling formalin (35 mL/m^3).

Glutaraldehyde is supplied as a 2% solution and is 'activated' before use by the addition of the bicarbonate compound supplied with the pack. The activated glutaraldehyde must be used within two weeks and discarded if any turbidity appears. Only glutaraldehyde should be used for instruments with a metal surface as hypochlorite causes corrosion.

8. Handling and Disposal of Contaminated Material and Waste

CONTAMINATED LABORATORY waste includes specimens, cultures, paper towels, tissues used to wipe benches, equipment and surfaces, disposable gloves and gowns, hypodermic needles and syringes, used pipettes, slides, coverslips, human/animal tissues, animal carcass, bedding from animal cages and liquids.

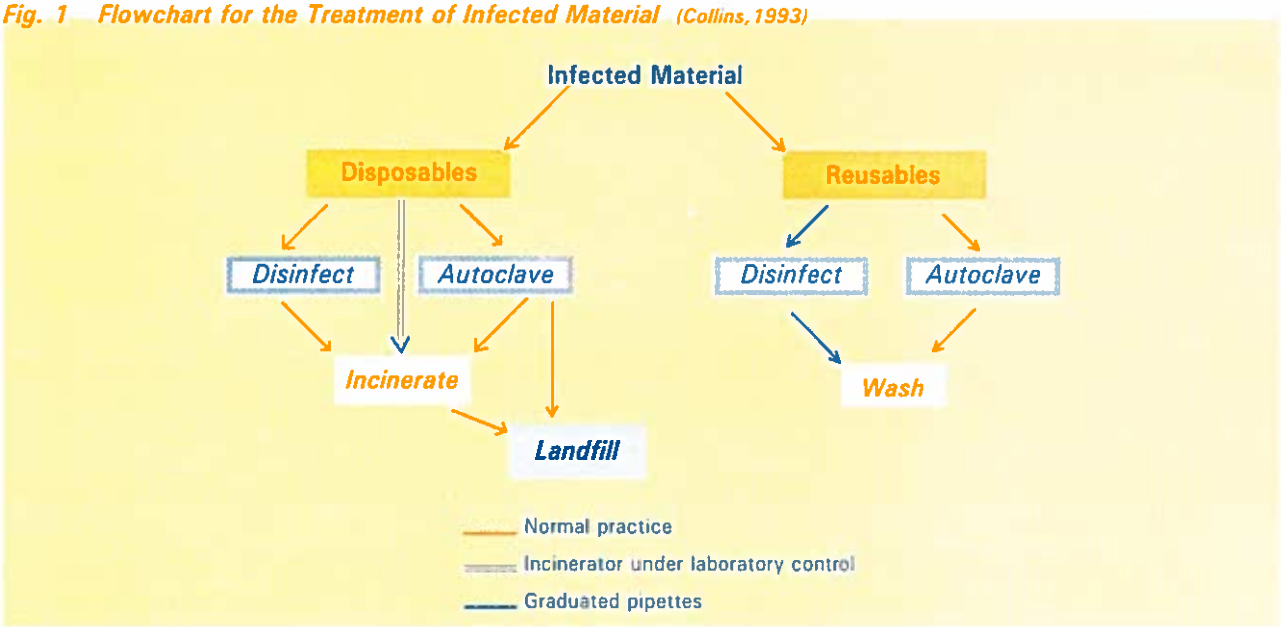
Decontamination depends on the nature of material. It is different for disposables and re-usables. The flowchart for these is given in Figure 1. Fluids and liquid contents of disinfectant discard jars are poured carefully down a sluice or deep sink without splashing. The exception is Risk Group 4 microorganisms.

Reusable equipment and specimen tubes should be placed in a puncture-resistant metal or plastic container and chemically disinfected prior to cleaning and being autoclaved or boiled. Gloves must be worn at all times during disinfection and cleaning. Contaminated protective clothing should be placed in a separate container located within the laboratory and autoclaved or disinfected and washed before reuse.

Disposable contaminated equipment should be placed in a puncture-resistant metal or plastic container and, together with other contaminated material, should be autoclaved, boiled or chemically disinfected in the work area. Alternatively, such material may be transported from the work area in a securely covered leakproof container to a central site on the laboratory premises for immediate autoclaving or incineration. If the containers are to be reused they should be cleaned and disinfected before reuse.

Incineration is the method of choice for disposal of contaminated material and waste if the incinerator is located on laboratory premises and under laboratory control. If the material has to be removed from the premises, it must first be autoclaved

Fig. 1 Flowchart for the Treatment of Infected Material (Collins, 1993)



or otherwise decontaminated. Ashes and debris should be buried in a landfill site.

When incineration is impossible, decontaminated material and waste should be disposed of in a controlled landfill. Care must be taken to

Incineration is the preferred method for disposal of contaminated material and waste.

ensure that all material and wastes have been sterilized or disinfected. Syringes and needles are destroyed mechanically. The material should be deposited in trenches, covered with earth and compacted daily. The controlled fill must be fenced off.

Radioactive material should not be incinerated; it should be disposed of in accordance with national codes and requirements.

9. Guidelines for the Handling, Transfer and Shipment of Specimens

HANDLING, TRANSFER and shipment of improperly packed specimens carry the risk of infection for all people engaged in these activities, from laboratory workers through administrative staff to postal personnel.

To avoid such risks:

- Specimen containers should be leak-proof, break-resistant, made of plastic or glass, and preferably have screw-caps.
- After the container is closed and sealed, it should be wiped with disinfectant and then dried.
- When a specimen is received and before the container is opened, it should be wiped with disinfectant and then dried.

Transport of Specimens by Public Conveyance

WHO, together with international agencies concerned with public transportation, has developed regulations for the shipment of specimens by mail, air freight and other common carriers.

These regulations are as follows:

- The specimen should be placed in a watertight receptacle of good-quality glass or plastic. The closure must be tight to prevent leaking and it must be held in position with wire, adhesive tape or other secure means.
- The specimen container should be wrapped in enough absorbent material (paper towels or tissue or absorbent cotton wool) to absorb all the fluid in case of leakage.
- The wrapped specimen container (or several containers) should be placed in a durable watertight container, and enough absorbent material must be used to cushion the specimen containers suitably within the second container.
- Within the health care facility and laboratory, specimen containers should be placed in racks to maintain them in an upright position. The racks should be transferred and transported in leak-proof containers that will contain accidental leakages or spillages.
- Specimen containers in racks being transported from field collection sites or between laboratories in laboratory-controlled vehicles should be in leak-proof boxes with secure, tight-fitting covers.
- Transport and storage in areas without a reliable electricity supply may require different approaches.
- The secondary container should be placed in a package strong enough to protect the contents from physical damage during transit.
- Specimen data forms, letters and other information that identify or describe the specimen should be taped to the outside of the secondary watertight container.
- National and international shipping and transport regulations must be observed.

10. Conclusion

BY FOLLOWING biosafety practices, laboratory personnel can protect themselves and prevent accidental spread of Risk Groups 3 and 4 microorganisms in the community. Biosafety measures must be observed while conducting laboratory investigations. Simple tests are performed by persons with minimal training in conventional laboratory procedures, who should be exhorted to observe the norms associated with working with infectious material. Furthermore, such tests are carried out in the field and at the patient's bedside and could involve inadvertent contact with Risk Groups 3 and 4 microbes. Any lapse in the dos and don'ts of biosafety could be disastrous. Laboratory personnel must face the challenge of the HIV pandemic by following good microbiological techniques, prompt management of accidents and spills and adequate disposal of materials soiled with hazardous and life-threatening pathogens.

Further Reading

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2. Furr AK (ed). *CRC handbook of laboratory safety*, 3rd ed. Boca Raton, Florida, CRC Press, 1991.
3. Collins CH. *Laboratory acquired infections*, 3rd ed. Oxford, Linacre House, Butterworth Heinemann Ltd., 1993.
4. *Guidelines for preventing HIV, HBV and other infectious in the health care settings*. New Delhi, India, World Health Organization; Regional Office for South-East Asia,

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About the Book

WHO priorities in the 1990s have been to improve and promote essential health technology applicable at the primary health care level and the immediate supporting level of the health system, to accelerate the process for Health for All by the Year 2000.

Proper surveillance with a sound and efficient network of laboratories is essential to meet the preventive, promotive, diagnostic, therapeutic and rehabilitative components of health care. At present, laboratory support at the primary health care level is not fully developed in most developing countries.

In order to achieve efficient and relevant primary health care, there is a need:

(1) to develop a national policy on the development of laboratory competence of the peripheral level; (2) to introduce appropriate diagnostic procedures for common communicable diseases; and (3) to ensure quality assurance of these procedures. These three aspects have been enunciated in detail in this book.

Appropriate guidelines have been given for the structure, management functions and scope of activities of health laboratories in countries in Part I of the book.

The impact of simple diagnostic technology will be felt in early diagnosis and therapeutic and control interventions of common diseases, reduction of disease burden and promotion of health in the communities. This aspect is discussed in Part II of the book.

The importance of introducing, managing and sustaining quality assurance in the countries has been emphasized in Part III. The principles and management of quality assurance programmes have been presented in detail for clinical chemistry, clinical microbiology, haematology and blood banks.

The phenomenal rise in the incidence of AIDS, hepatitis, tuberculosis and other emerging infectious poses increased risk for laboratory workers handling the clinical specimens. To safeguard the laboratory personnel, the chapter on 'biosafety in the laboratories' has been revised and updated and included as chapter IV.

World Health Organization
Regional Office for South-East Asia
New Delhi